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RADIAL OSCILLATIONS OF THE LIMITING MODELS OF POLYTROPIC GAS SPHERES

By ZDENĚK KOPAL

CENTER OF ANALYSIS, MASSACHUSETTS INSTITUTE OF TECHNOLOGY AND HARVARD COLLEGE OBSERVATORY

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A gas sphere is said to be in polytropic equilibrium if the relationship between the pressure P and density ρ at any point in its interior is expressed as

$$P = K\rho^{1+1/n} \tag{1}$$

where K and n are constants. If we introduce, as usual, the non-dimensional variables θ and ξ related with the density ρ at a distance r from the center by

$$r = \left[\frac{(1+n)P_c}{4\pi G\rho_c^2}\right]^{1/2}\xi \text{ and } \rho = \rho_c\theta^n, \tag{2}$$

where the subscript c denotes the central values of the respective quantities and G stands for the gravitational constant, the new function θ describing the distribution of density in the interior of a polytropic gas sphere under the influence of its own gravity is governed by Emden's well-known equation

$$\frac{1}{\xi^2}\frac{d}{d\xi}\left(\xi^2\frac{d\theta}{d\xi}\right) + \theta^n = 0. \tag{3}$$

If the mass of a polytrope is to be finite, it can be shown that $0 \leqslant n \leqslant 5$. For both limiting cases, equation (2) admits of a solution in closed form: namely, if $n = 0$, the solution of (3) which is regular at the center becomes

$$\theta = 1 - \frac{1}{6}\xi^2, \tag{4}$$

whereas if $n = 5$,

$$\theta = \left(1 + \frac{1}{3}\xi^2\right)^{-1/2}. \tag{5}$$

The former case refers to a gas sphere the density of which is constant throughout the interior; the latter, to a sphere of finite central density but of infinite radius and therefore vanishing mean density.

The object of the present paper is to investigate what happens when a polytropic gas sphere characterized by $n = 0$ or $n = 5$ is disturbed from its state of equilibrium by an instantaneous impulse in such a way that a small purely radial motion results. If the corresponding displacement $\delta\xi$ of ξ occurs adiabatically, and is so small that squares and higher powers of its amplitude can be ignored, Eddington[1] has shown that the Lagrangian hydrodynamical equations can be reduced to the following second-order differential equation

$$\frac{d^2f}{d\xi^2} + \frac{4 - \mu}{\xi}\frac{df}{d\xi} + \left\{\frac{\omega^2}{\theta} - \frac{\alpha\mu}{\xi^2}\right\} f = 0, \tag{6}$$

where $f \equiv \delta\xi/\xi$ and

$$\left.\begin{aligned} \mu &= -(n+1)\frac{\xi}{\theta}\frac{d\theta}{d\xi}, \\ \alpha &= 3 - \frac{4}{\gamma}, \\ \omega^2 &= \frac{(n+1)\nu^2}{4\pi G\gamma\rho_c}, \end{aligned}\right\} \tag{7}$$

$2\pi/\nu$ being the period of the respective oscillation and γ, the ratio of specific heats. The boundary conditions of our problem which require that there be no displacement at the center and no variation of pressure on the surface are formally equivalent to a requirement that equation (6) have regular integrals that are finite for $0 \leqslant \xi \leqslant \xi_1$, where ξ_1 denotes the first zero of the respective Emden function.

Polytrope $n = 0$.—Consider first a compressible gas sphere in which the undisturbed density is constant and $\xi_1 = \sqrt{6}$. Setting $\xi/\xi_1 = x$ and making use of (4) we find that $\mu = 2x^2/(1 - x^2)$ and equation (6) assumes the form

$$(1 - x^2)\frac{d^2f}{dx^2} + \frac{4 - x^2}{x}\frac{df}{dx} + Jf = 0, \tag{8}$$

where we have abbreviated

$$J = 6\omega^2 - 2\alpha. \tag{9}$$

A substitution $x^2 = y$ transforms (8) into a hypergeometric equation

$$y(1-y)\frac{d^2f}{dy^2}+[c-(a+b+1)y]\frac{df}{dy}-abf=0, \qquad (10)$$

where

$$a, b=\frac{5}{4}\pm\sqrt{\left(\frac{5}{4}\right)^2+\frac{1}{4}J} \qquad (11)$$

and $c = 5/2$.

The particular solution of (10) which remains regular at the origin is

$$f=AF\left(a, b, \frac{5}{2}, x^2\right), \qquad (12)$$

where A is an arbitrary constant. The second requirement of our boundary conditions, that f be finite for $x^2 = 1$, discloses that the hypergeometric series on the right-hand side of (12) must either converge or—if divergent—must be terminating. In our present problem $a + b = c$, in which case the hypergeometric series is known to diverge for $x = 1$.[2] Hence, if (12) is to represent a physically admissible solution of our problem, the series on the right-hand side of (12) must reduce to a polynomial—which means that a or b must be equal to some negative integer. If we assume that $b = -j$ (an assumption of $a = -j$ would lead to exactly the same consequences), where j is zero or a positive integer, it follows from (11) that

$$J=2j(2j+5). \qquad (13)$$

Therefore, a homogeneous gas sphere, disturbed slightly from its state of equilibrium, is found to be able to oscillate radially, not in any frequency, but only in discrete frequencies given by

$$\nu^2=\frac{4}{3}\pi G\rho_c\gamma\{j(2j+5)+\alpha\}, \qquad (14)$$

which are real provided that $J + 2\alpha > 0$, i.e., that

$$\gamma>\frac{4}{j(2j+5)+3}. \qquad (15)$$

The corresponding eigenfunctions (12) of the radial motion reduce them to the Jacobi polynomials[3]

$$f=AG_j\left(\frac{5}{2}, \frac{5}{2}, x^2\right), \qquad (16)$$

while the corresponding variation δP of pressure P accompanying the radial oscillation is given by

$$\frac{\delta P}{P} = -\frac{\gamma}{x^2}\frac{d}{dx}(x^3 f) = -3\gamma A G_j\left(\frac{5}{2}, \frac{3}{2}, x^2\right). \qquad (17)$$

It should be mentioned that the discrete nature of the frequency spectrum of radial oscillations of a homogeneous configuration of compressible gas was previously pointed out by Sterne;[4] in point of fact, its fundamental period (corresponding to $j = 0$) was first found by Miller.[5] Both these investigators failed, however, to recognize the hypergeometric character of the equation (8), or the fact that the proof of the discrete nature of the spectrum is based on the divergence of a hypergeometric series of unit radius, and that the respective eigenfunctions of the oscillation in radius or pressure are Jacobi polynomials.

Polytrope $n = 5$.—Let us now turn to the other limiting case of the polytropic family of models—that involving an infinite degree of central condensation in which $n = 5$. From the astrophysical point of view, this case is of much greater interest than a homogeneous configuration since the internal density concentrations in real stars are known to be high and, therefore, the properties of a polytrope $n = 5$ may, in certain respects, come close to those of actual stars.

In order to investigate the possibility of small radial oscillations of a gas sphere built up according to this model, we return to Schuster's solution (5) of Emden's equation (3) for $n = 5$ and introduce a new independent variable τ defined by

$$\xi = \sqrt{3}\tan\tau. \qquad (18)$$

From this it is easily demonstrated that $\theta = \cos\tau$ and $\mu = 6\sin^2\tau$. Assuming moreover,

$$f = \frac{v(\tau)}{\sin^2\tau\cos^2\tau}, \qquad (19)$$

Eddington's equation (6) governing small radial oscillations of polytropic stars takes the explicit form

$$\frac{d^2v}{d\tau^2} + 2\left\{8 - \frac{1}{\sin^2\tau} + \frac{2}{\cos^2\tau}\left(\frac{6}{\gamma} - 5\right) + \frac{3\omega^2}{2\cos^5\tau}\right\}v = 0. \qquad (20)$$

Before proceeding with the solution of this equation, let us make more precise the physical significance of the model we are considering. The polytrope $n = 5$ represents a gas sphere of finite mass and central density, but of infinite extent. With certain precautions such a configuration can, however, be converted into one of finite mass and dimensions, but of infinite central density. In more specific terms, the mass m and radius r of the polytrope $n = 5$ are known[6] to be

$$\left.\begin{aligned} m(\tau) &= 4\sqrt{3}\,\pi\left(\frac{3K}{2\pi G}\right)^{3/2}\rho_c^{-1/2}\sin^3\tau, \\ r(\tau) &= 3\left(\frac{K}{2\pi G}\right)^{1/2}\rho_c^{-2/3}\tan\tau, \end{aligned}\right\} \tag{21}$$

with boundary at $\tau = 90°$. From these equations it is obvious that the only way to obtain finite dimensions of the whole configuration for $K \neq 0$ is to make ρ_c infinitely large. A polytrope $n = 5$ is, however, seen to possess the curious feature that its mass tends toward zero, for finite values of K, when its central density increases beyond any limit. The mass can, therefore, remain finite only provided that ρ_c and K *both* approach infinity in such a way that

$$K^{3/2}/\rho_c^{1/2} = \text{constant}. \tag{22}$$

The radius r then will, by definition, be a finite limit of the indefinite product $\rho_c^{1/2}\cos 90°$; the reader can easily verify that this definition is sufficient to make the pressure vanish at the boundary of our configuration.

If the foregoing conditions are met, it is apparent that an assumption of infinite central density can effect a homologous transformation of the polytrope $n = 5$ into a configuration of finite dimensions, the whole mass of which is confined to its center and surrounded by an envelope of negligible weight, in which the density falls off as the inverse fifth power of the distance from the center. Equations (7) make it evident, however, that, under these circumstances, *the value of ω^2 is found to be zero for any finite frequency ν of free radial oscillation of the respective configuration.* In virtue of this fact, the term of the right-hand side of (20) multiplied by ω^2 drops off, and the remaining equation proves again to be one of the hypergeometric type.

Changing over from τ to a new independent variable $x = \sin^2\tau$ and taking

$$v(\tau) = \eta(x)\sin^m\tau\cos^n\tau, \tag{23}$$

where

$$m(m-1) = 2, \tag{24}$$

$$n(n-1) = 4(5 - 6/\gamma) \tag{25}$$

equation (20) can be reduced to

$$x(1-x)\frac{d^2\eta}{dx^2} + [c - (a+b+1)x]\frac{d\eta}{dx} - ab\eta = 0, \tag{26}$$

where now

$$\left.\begin{aligned} a &= \frac{1}{2}(m + n + 4), \\ b &= \frac{1}{2}(m + n - 4), \\ c &= \frac{1}{2} + m, \end{aligned}\right\} \tag{27}$$

and combining its general solution with (19) and (23) it follows that

$$f = \sin^{m-2}\tau \cos^{n-2}\tau \left\{A F(a, b, c, x) + Bx^{1-c} F(a + 1 - c, b + 1 - c, 2 - c, x\right\}, \tag{28}$$

where A and B are integration constants.

The boundary conditions of our problem require f to remain finite for $x = 0$ and 1. The condition at the origin asserts at once that $B = 0$ and that, of the two roots of equation (24), only $m = 2$ is admissible; for then $f(0) = A$ which, without the loss of generality, can be set equal to one. This leaves us with

$$f = (1 - x)^b F\left(a, b, \frac{5}{2}, x\right) \tag{29}$$

as a possible solution of our physical problem, where $a = {}^1/_2\,(n + 6)$ and $b = {}^1/_2\,(n - 2)$, n being a root of equation (25) whose value depends on γ.

If the pressure is to remain constant over the free surface of our radially oscillating configuration, f must retain a finite value at $x = 1$. The hypergeometric series on the right-hand side of equation (29) will again diverge or converge for $x = 1$ as $a + b \gtrless c$, i.e., as n is greater or smaller than one half. An inspection of equation (25) reveals that, for any value of $\gamma > {}^{32}/_{27}$, the equation admits indeed of a pair of roots one of which is always less than 0.5, so that for every value of γ greater than this limit a value of n can be found which will make our hypergeometric series converge. However, if $n < 0.5$, then $b < -0.75$, and therefore the binomial $(1 - x)^b$ factoring the hypergeometric series on the right-hand side of equation (29) will again become infinite for $x = 1$. The values of b (or n) for which the product on the right-hand side of (29) may remain finite can easily be found if we write it in the form $F(a, b, c, x) \div (1 - x)^{-b}$ and observe that, whereas the hypergeometric series in the numerator can be written as

$$1 + \frac{ab}{1!c}x + \frac{a(a + 1)b(b + 1)}{2!\ c(c + 1)}x^2 + \ldots,$$

a binomial expansion of the denominator yields

$$1 + \frac{b}{1!}x + \frac{b(b + 1)}{2!}x^2 + \ldots.$$

We see that both series become identical if (1) $b = 0$ (i.e., $n = 2$),(2)$a = c = {}^5/_2$ (i.e., $n = -1$). Either case corresponds to the ratio of specific heats $\gamma = {}^4/_3$ and the equilibrium of our configuration is clearly neutral. For any other value of γ—greater or smaller than ${}^4/_3$—the amplitude of the radial oscillations becomes, however, infinite at $x = 1$, so that the boundary condition on the surface cannot be met. The conclusion follows, therefore, that a gaseous configuration of matter in hydrostatic equilibrium, obtained by a homologous transformation of a polytrope $n = 5$ into a sphere of finite dimensions, is incapable of exhibiting radial oscillations of finite frequency or amplitude.

It is a known fact that free periods of radial oscillations of polytropic gas spheres decrease, in general, with increasing degree of central condensation.[7] Free periods of various modes of the polytrope $n = 0$ can, therefore, be regarded as upper bounds of the respective quantities for the polytropic family of models. On the other hand, when $n = 5$, no free periods were found to exist; their lower bound for the polytropic family of models therefore appears to be zero. It should be stressed, however, that the non-existence of finite periods of free oscillation of the polytrope $n = 5$ is *not* a consequence of the fact that the ratio of its central density to the mean density is infinite, but rather of the *rate* at which the density diminishes with increasing distance from the center. Sterne[4] has, in fact, investigated another model, characterized by infinite central condensation, in which the density falls off as the inverse square of the distance from the center; and this model was found to admit of a whole spectrum of discrete periods of radial oscillations. On the other hand, the polytrope $n = 5$, for which the density in the envelope falls off approximately as inverse fifth power of the distance from the center, proved incapable of such oscillations. It appears therefore, that for spherically symmetrical configurations of compressible gas, the whole mass of which is confined to the center and is surrounded by an envelope which does not contribute appreciably to the gravitational potential within the body—a configuration rather loosely termed in the literature as the Roche model—a certain critical value of the density gradient in the envelope must exist beyond which radial oscillations are no longer possible. This critical value of $(r/\rho)\,(d\rho/dr)$ must, moreover, be between -2 and -5. Sen[8] claimed recently to have proved that, if the density in the envelope is assumed to vary as r^{-p}, where p is a positive integer, a failure of the convergence of a series expansion of the solution of the governing differential equation implies the non-existence of any permissible frequency of radial oscillation of the respective configuration if $p > 3$. Although the correctness of a part of Sen's investigation was challenged by Cowling,[9] Sen's criterion appears to be consistent with the particular result established above for the polytrope $n = 5$. A generalization of Sen's work for non-integral values of p and a consequent clarifica-

tion of the limit of instability of the Roche model for radial oscillations are much to be desired.

[1] *M. N.*, **79**, 2 (1918); or *Internal Constitution of the Stars*, Cambridge, 1930, pp. 186–188. In his papers Eddington gave the explicit form of the equation (6) corresponding to the polytropic index $n = 3$ ("standard model"). The general form of equation (6) for unrestricted n was first explicitly stated by Miller, *M. N.*, **90**, 59 (1929).

[2] Cf., for instance, Bromwich, *Introduction to the Theory of Infinite Series*, Cambridge, 1908, p. 35.

[3] In the notations of Courant-Hilbert, *Methoden der Mathematischen Physik*, Berlin, 1931, vol. I, p. 77.

[4] *M. N.*, **97**, 582 (1937).

[5] *Ibid.*, **90**, 59 (1929).

[6] Cf., for instance, Chandrasekhar, *An Introduction to the Study of Stellar Structure*, Chicago, 1939, p. 97.

[7] Cf. Miller, op. cit., the same fact holds true for non-radial oscillations as well (Cowling, *M. N.*, **101**, 367, 1942).

[8] *Proc. Nat. Acad. Sci., India*, **A12**, 99 (1942).

[9] *Math. Reviews*, **8**, 60 (1947).

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*EFFECTS OF DIFFERENT PROTEIN AND RIBOFLAVIN CONTENTS OF DIET UPON THE CHEMICAL COMPOSITION OF THE BODY**

BY HENRY C. SHERMAN AND MILDRED S. RAGAN†

DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY

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It has been shown that storage of riboflavin in the liver of the rat not only is correlated with the riboflavin intake[1] but also tends to increase with the protein content of the diet.[2–6] Some experiments have also shown increased storage of protein, with or without decrease of fat, in the body at increased levels of nutritional intake of riboflavin.[7–9] The present paper reports experiments upon the effects of varying both protein and riboflavin intakes upon the protein and riboflavin contents of the whole carcass as well as of the liver.

Thirty-day-old rats from the colony maintained in this laboratory were used as the experimental animals, and the period of experimental feeding was 30 days. The paired feeding method was used. The rats on the low protein diet were restricted to the amount of food eaten by those on the high protein, and those on high riboflavin were restricted to the amount eaten by those on the low riboflavin diet. Litter-mates matched as to sex were used on the diets being compared.

As seen in table 1, increasing the protein content from 12 to 32 per cent, in a diet containing 7 μg. of riboflavin per gram of air-dry food, resulted in

TABLE 1

EFFECT OF INCREASING PROTEIN IN A DIET OF LIBERAL RIBOFLAVIN CONTENT: 7 μG. PER GRAM

(Average of 6 comparisons)

FINDINGS	WITH 12% PROTEIN DIET	WITH 32% PROTEIN DIET
Gain in body weight, g.	51.	68.
Liver weight, g.	5.94	7.88
Nitrogen in carcass, %	3.12	3.39
Nitrogen in liver, %	2.75	3.01
Riboflavin in carcass, μg.	4.93	5.49
Riboflavin in liver, μg./g.	28.1	31.9
Total nitrogen in body, g.	2.78	3.44
Total protein in body, g.	17.3	21.5
Total protein in body, %	16.95	18.42
Riboflavin per g. rat, μg.	5.70	6.63
Total riboflavin in body, μg.	631.	824.

ncreased growth and liver weight; and more growth per gram of food consumed, though the percentage utilization of protein was lower on the diet of higher protein content. On the higher protein diet the body retained more riboflavin, more protein, less fat and more water.

Table 2 shows the results of corresponding experiments with three diets containing uniformly 12 per cent protein but with 1, 3 and 9 μg. of ribo-

TABLE 2

EFFECT OF INCREASING RIBOFLAVIN IN A 12% PROTEIN DIET

(Average of 5 Comparisons)

FINDINGS	WITH 1 μG. OF RIBOFLAVIN/G.	WITH 3 μG. OF RIBOFLAVIN/G.	WITH 9 μG. OF RIBOFLAVIN/G.
Gain in body weight, g.	35.	38.	41.
Liver weight, g.	4.81	4.74	4.81
Nitrogen in carcass, %	2.91	2.92	2.95
Nitrogen in liver, %	2.84	2.85	2.87
Riboflavin in carcass, μg./g.	5.19	5.53	6.56
Riboflavin in liver, μg./g.	20.28	26.00	30.50
Total nitrogen in body, g.	2.23	2.26	2.71
Total protein in body, %	16.66	16.93	17.22
Riboflavin per g., μg.	6.11	6.95	8.14
Total riboflavin in body, μg.	468.	544.	632.

flavin per gram of air-dry food, respectively. Here the increase of riboflavin intake clearly increased the riboflavin content both of the carcass and of the liver, and both the amount of riboflavin per gram of body weight and

the total weight of riboflavin in the body; while the percentages of nitrogen were not significantly changed, either in the carcass or the liver, or in the body as a whole, by these differences of riboflavin intake at this 12 per cent level of food protein.

The same three levels of riboflavin intake were then tested with a different basal ration of moderately higher protein content—viz., 20 per cent of protein in the air-dry food mixture—with the results shown in table 3. Here the increases of intake of riboflavin had no significant influence upon the growth of the body or the amount of nitrogen it retained, either in carcass or liver; but definitely increased the riboflavin content of the liver and of the body as a whole. The percentage of total protein in the body as a

TABLE 3

EFFECT OF INCREASING RIBOFLAVIN IN A 20% PROTEIN DIET

(Average of 5 Comparisons)

FINDINGS	WITH 1 μG. OF RIBOFLAVIN/G.	WITH 3 μG. OF RIBOFLAVIN/G.	WITH 9 μG. OF RIBOFLAVIN/G.
Gain in body weight, g.	40.	43.	41.
Liver weight, g.	5.45	4.79	4.82
Nitrogen in carcass, %	3.39	3.23	3.30
Nitrogen in liver, %	3.15	3.07	3.00
Riboflavin in carcass, μg./g.	5.08	5.08	5.50
Riboflavin in liver, μg./g.	17.85	24.38	29.06
Total nitrogen in body, g.	2.45	2.54	2.57
Total protein in body, %	18.00	18.20	18.60
Riboflavin per g., μg.	6.04	6.67	7.17
Total riboflavin in body, μg.	438.	511.	537.

whole showed a very slight trend toward increase at the successively higher levels of riboflavin intake, consistently with the well-known "concentration" or "mass action" principle and the protein-riboflavin relationship which is being established by current research; but the measured differences are too small to be statistically significant, so that the effect of the protein-riboflavin relationship upon protein retention in the body is practically plateaued at the intake levels of these experiments.

In all of the above-described experiments, the "paired-feeding" method was used. *Ad libitum* feeding was also used in some cases with the following results: In these experiments as in those above, increased nutritional intake of riboflavin, within the range 1 to 9 μg. per gram of food, tended to increase the amounts stored in the body, both when the protein level was 12 and when it was 20 per cent of the air-dry food. The diet of highest riboflavin content was eaten in largest amount and resulted in largest gains of body weight and of riboflavin content of body; but the protein-riboflavin relationship was less clearly shown with *ad libitum* than with "paired" feeding.

Summary.—Paired-feeding experiments showed an increase of both riboflavin and protein content of liver and carcass on a diet containing 7 μg. of riboflavin per gram when the protein content of the diet was increased from 12 to 32 per cent.

Increasing the riboflavin content of a 12 per cent protein diet from 1 to 9 μg. per gram resulted in slight increase in weight, in fat and in total protein stored. The riboflavin in liver and carcass was also increased.

The same increase, from 1 to 9 μg. of riboflavin per gram of air-dry food, on a 20 per cent protein diet had only slight influence upon growth or nitrogen stored, but increased the storage of riboflavin.

When food was allowed *ad libitum*, increasing the riboflavin in a 20 per cent protein diet from 1 to 9 μg. per gram resulted in increased appetite, growth and retention of protein and riboflavin, though the percentages of protein were less because of the greater gain in body weight.

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† Present address: Food and Nutrition Board, National Research Council, 2101 Constitution Avenue, Washington, D. C.

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*ON THE FREQUENCY AND TRANSMITTED CHROMOSOME ALTERATIONS AND GENE MUTATIONS INDUCED BY ATOMIC BOMB RADIATIONS IN MAIZE**

BY E. G. ANDERSON

KERCKHOFF LABORATORIES OF BIOLOGY, CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, CALIFORNIA

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Samples of maize seed of the single cross L289 × I205 were placed on some of the ships within the target area at Bikini at the time of the atomic

bomb test of July 1, 1946. At about the same time Dr. L. F. Randolph exposed duplicate samples to x-rays at Cornell University. Plantings were made at the California Institute of Technology of both series together with untreated controls. From these plants, sporocyte samples were collected by Dr. Randolph for studies at Cornell University on the visible chromosomal changes induced by the radiations (Randolph, Longley and Li *in press*). Studies on hereditary effects have been carried on at the California Institute of Technology in coöperation with the United States Department of Agriculture and the Naval Medical Research Section of Operation Crossroads.

Seedling comparisons supplemented later by pollen sector frequencies indicated one lot of seed exposed to atomic bomb radiations has received a dosage equivalent in biological effects to slightly below 15,000 r units of x-ray. Most of the studies have been made on this lot and on the comparable lot exposed to 15,000 r units x-ray.

In maize, chromosomal alterations can be detected readily by partial pollen sterility, unless the alteration involves only a very small portion of a chromosome. Examination of 751 plants of the unexposed controls showed only normal pollen.

Plants from irradiated seeds are mosaics of various sectors, each of which is assumed to be the progeny of a single meristematic cell of the multicellular growing tip of the embryo. Observations on the pollen indicated that about 90 per cent of the tassels of the Bikini lot had one or more sectors showing chromosome alterations.

Three plants were outcrossed to normals or to plants of low dosage when normal plants were not available. The outcross plants were derived from one normal gamete and one from the irradiated source, and should thus give a direct frequency of such transmissible effects as could be detected or recognized by the techniques employed.

In order to obtain a large number of chromosomal alterations for cytological study, ten seeds of each outcross were planted. The number of partial-sterile plants obtained was expected to be a little higher than random, since

1. Pollen was taken only from plants known to have at least one sector of partial sterility,

2. The majority of these pollinations were made on low dosage lots rather than on normal controls,

3. In the outcrosses of the Bikini or x-ray lots as female parents, seed was selected from poorly filled portions of the ear. Such parts are most likely to be partial-sterile sectors.

The observed frequencies were

	TOTAL PLANTS	PER CENT PARTIAL-STERILE
Normal or low dosage × Bikini pollen	3176	4.7
Normal or low dosage × 15,000 r x-ray	2582	6.0
Bikini × normal pollen	5456	6.4
15,000 r x-ray × normal pollen	3248	7.8

An unselected sample should give a somewhat lower frequency of transmitted partial-sterile plants, probably 4.5 or 5.0 per cent.

All partial-sterile plants were outcrossed to standard stocks to establish lines for cytogenetic study.

Some of the normal plants were self-pollinated to obtain mutant characters for other studies. In all, 1231 self-pollinated ears were harvested from the Bikini lot, and 655 from the corresponding 15,000 r x-ray lot. Of these 26 per cent of the ears from the Bikini lot and 23 per cent of those from x-ray were tabulated as segregating for endosperm characters such as white endosperm, sugary, brittle, opaque, shrunken, many types of defective endosperm and also several viviparous types. No doubt many defective types were overlooked.

Seedling tests are in progress and a test for mature plant characters will be made. Conspicuous seedling characters have been found to be even more frequent than the endosperm defects. The seedling characters include chlorophyll deficiencies such as white (albino), pale yellow and yellow lethals, pale green and yellow-green seedlings, virescents, piebald, zebra, striate and striped seedlings. Some appear to be mutable. A wide range of other characters such as glossies, dwarfs and a host of morphologically aberrant types have also appeared, many of which are unlike any described in the maize literature.

Because of the difficulty of establishing any objective standards for detecting and classifying miscellaneous mutations, no plans had been made for studies on mutation frequency and accordingly no controls were self-pollinated to test for the presence of recessive gene defects in the stock used. Some recessives could be carried in the commercial inbred lines, L289 and I205, used in making up the single cross, but not in such frequencies as were obtained. A number of control plants were used as pollen parents in a series of outcrosses with no clear evidence of any recessive having been carried in the control parent. Whenever the source could be determined the recessive came from the irradiated parent. It is possible that a minor portion of the gene defects may have been present in the stock, but at most it could have been only a small fraction. Additional tests will be made the coming summer including a study of the lower dosages and a checkup of the controls.

From data thus far obtained, it appears that most of the outcross plants carried one or more recessive mutations from the irradiated parent. Thus more than 50 per cent of the functioning gametes of plants from seed exposed to 15,000 r units x-ray or to the nearly equivalent atomic radiation exposure carry one or more gene mutations caused by the radiations.

The dormant seeds in these tests were exposed to much greater irradiation than the lethal dose for most animals or for actively growing plant tissue. But roughly similar frequencies obtained from 15,000 r x-ray and the nearly equivalent Bikini lot support the expectation that atomic bomb radiations have about the same heritable effects as comparable doses of x-rays.

Summary.—Maize seed receiving atomic bomb radiations equivalent to nearly 15,000 r units x-ray were tested for hereditary chromosomal alterations and gene mutations.

The frequency of chromosomal alterations as indicated by partial pollen sterility was 4.7–6.4 per cent in progenies which were not entirely random. It is estimated that a random frequency would be between 4.5 and 5.0 per cent.

The frequency of gene mutation was extremely high. Probably more than 50 per cent of the gametes of the exposed generation carried one or more gene mutations.

The frequencies of chromosomal alterations and gene mutations in a lot exposed to 15,000 r units x-ray were roughly equivalent to the frequencies obtained in the Bikini lot.

* Coöperative investigations of the Kerckhoff Laboratories of Biology, California Institute of Technology, and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture.

This is a brief report based on a coöperative program carried on at the California Institute of Technology by a group of investigators including Dr. A. E. Longley of the United States Department of Agriculture, Prof. E. F. Frolik of the University of Nebraska, Dr. E. E. Dale of Union College and the following graduate student assistants: C. H. Li, K. L. Retherford, Earl P. Patterson, Wayne F. Keim and Don Robertson.

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*THE RELATION BETWEEN NICOTINIC ACID AND CARBOHYDRATES IN A SERIES OF MAIZE ENDOSPERM GENOTYPES**

BY JAMES W. CAMERON AND H. J. TEAS†

UNIVERSITY OF CALIFORNIA CITRUS EXPERIMENT STATION, RIVERSIDE, AND KERCKHOFF LABORATORIES OF BIOLOGY, CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA

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It has been shown by Burkholder, McVeigh and Moyer,[1] by Barton-Wright,[2] and by Mather and Barton-Wright[3] that kernels of surgary maize

are about twice as high in nicotinic acid as those of starchy maize. In the first-mentioned paper, kernels of a group of sugary strains were reported to average 34.6 micrograms of nicotinic acid per gram of tissue, as contrasted with 20.8 for starchy strains; in the last two papers the averages were 26.2 for sugary strains and 15.8 for starchy. The hereditary differences in the carbohydrates in all these strains were presumably controlled by the allelic genes Su_1 and su_1, which act in such a way that kernels homozygous for the recessive su_1 are sugary, while those carrying one or more doses of Su_1 are starchy. No studies of the products of controlled crossing between Su_1 and su_1 strains were reported, but Mather and Barton-Wright[3] analyzed starchy and sugary kernels from open pollination on ears of five sugary lines. These kernels, whose triploid endosperms were of the genotypes $Su_1su_1su_1$ and $su_1su_1su_1$, respectively, showed essentially the same difference in nicotinic acid content as that cited above. (Triploidy is normal in the maize endosperm, owing to fusion of two female polar nuclei with one male gamete.)

Cameron[4] studied the effects of two pairs of alleles, su_1^{am} *su* and *Du du*, on the reserve carbohydrates in a series of maize endosperm genotypes. The gene su_1^{am} is a member of the Su_1su_1 series (for convenience, subscript numerals for this allelic series are omitted hereafter); whereas *Du* and *du* are wild type and recessive forms at an independent locus.[5] These genes interact so that endosperms carrying one or more doses of both su^{am} and *Du* are primarily starchy type (opaque and smooth) in appearance, while those homozygous for *su* or *du*, or both, are sugary type (translucent and wrinkled). Chemical analyses, which provide a more sensitive measure of the genetic effects, showed that the homozygous double-recessive genotype *su su su du du du* was the lowest in starch and the highest in soluble carbohydrates and that starch increased and soluble carbohydrates decreased in a rather definite pattern with successive doses of either su^{am} or *Du*. First doses were usually the most effective, su^{am} was more effective than *Du*, and one dose of each gene was usually more effective than three doses of either alone. The behavior of the entire series of genotypes with respect to starch may be seen in table 1. Notations in the dosage column of this table make clear the identity of each genotype in terms of its gene dosage: 1–0 for example, carries one dose of su^{am} and none of *Du*, and therefore two doses of *su* and three of *du*.

The present paper reports data on the nicotinic acid content of this genotypic series, together with exploratory data on the thiamin and biotin content of selected genotypes.

Methods.—Mature maize endosperms from the same or sister ears of the series which had served for the carbohydrate studies[4] were used for the present analyses. The ears were produced in a single plot in one season,

TABLE 1

NICOTINIC ACID AND STARCH IN MATURE MAIZE ENDOSPERM GENOTYPES RESULTING FROM COMBINATIONS OF THE TWO GENE PAIRS *su^{am} su* AND *Du du*

GENERAL PHENOTYPIC CLASS	GENOTYPE	GENE DOSAGE, *su^{am}Du*	PER CENT STARCH* (DRY-WEIGHT BASIS)	MICROGRAMS NICOTINIC ACID PER GRAM OF TISSUE (AIR-DRIED)		
				INDIVIDUAL GENOTYPE	SUBGROUP MEAN	GROUP MEAN
Primarily starchy (opaque and smooth)	*su^{am}su^{am}su^{am} DuDuDu*	3–3	80.7	21.7	22.2	
	su^{am}su^{am}su^{am} DuDudu	3–2	77.7	22.3		
	su^{am}su^{am}su DuDuDu	2–3	76.0	21.4		
	su^{am}su^{am}su DuDudu	2–2	79.6	23.5		
	su^{am}su^{am}su^{am} Dududu	3–1	72.7	22.4		24.2
	su^{am}su^{am}su Dududu	2–1	71.8	29.4		
	su^{am}su su DuDuDu	1–3	69.7	22.2	25.8	
	su^{am}su su DuDudu	1–2	70.3	25.5		
	su^{am}su su Dududu	1–1	58.6	29.4		
Sugary (translucent and wrinkled)	*su^{am}su^{am}su^{am} dududu*	3–0	51.5	44.3	47.4	50.5
	su^{am}su^{am}su dududu	2–0	42.0	46.2		
	su^{am}su su dududu	1–0	33.6	51.6		
	su su su DuDuDu	0–3	32.2	56.3		
	su su su DuDudu	0–2	30.0	48.2	51.6	
	su su su Dududu	0–1	27.2	50.4		
	su su su dududu	0–0	19.2	56.7	56.7	

* Data from Cameron.[4]

with the exception of the genotype *su*^{am} *su su du du du*, which was grown in a different year.

Vitamin values are reported on an air-dry basis. Since earlier measurements had shown the entire range in moisture content to be from 7.6 to 10.5%, little error in relative values is introduced. Endosperms from each of two ears of each genotype were pooled and ground to 60 mesh in a Wiley electric mill, and the resulting material was thoroughly mixed. For the nicotinic acid assays reported in table 1, 1.0 g. of each sample was hydrolyzed by autoclaving with 50 cc. of 1 *N* sulfuric acid for 30 minutes at 15 lbs. pressure, and the suspensions were neutralized, filtered and made up to volume. The growth of *Lactobacillus arabinosus*, as determined by acid production during 72 hours at 35°C., was taken as a measure of the nicotinic acid. The culture medium and the general procedure were those described by The Association of Vitamin Chemists, Inc.[6] Calculations of nicotinic acid were made from the averages of three or more assays (each in duplicate) which agreed within 10% of the total average of duplicates representing six concentrations of added endosperm hydrolyzate. A sample of enriched wheat flour obtained from The Association of Vitamin Chemists, Inc., was assayed as a control in two experiments. The values obtained were within 3% of the range already reported by the Association (in personal communication to the junior author) for this material.

For the thiamin and biotin determinations certain strains of Neurospora were used, their response being measured by the dry weights of mycelial pads formed in a 72-hour growth period in liquid medium. The general techniques of culturing Neurospora strains, the means of supplying supplement and other procedures have been described.[7] Strain 18558, a thiamine-less mutant which requires vitamin thiazole or intact thiamin,[8] was used for the thiamin estimations. The biotin estimation was carried out with wild type strain 1*A*, which is deficient in the ability to synthesize biotin. The maize hydrolyzates for the biotin assay were the same as those used for nicotinic acid determinations. The samples for thiamin assay were prepared by simultaneous takadiastase and papain treatment in acetate buffer, according to the method of Cheldelin, *et al.*[9] For each determination a standard curve was run in duplicate, and five levels of endosperm hydrolyzate were used. The average of all values read from the relatively straight-line portion of the curve was taken in calculating the vitamin levels.

Results.—In table 1 the nicotinic acid content of the genotypes is shown in relation to the previously determined values for starch.[4] Whereas the starch content had shown a stepwise response to gene dosage, ranging from 19.2% starch in genotype 0–0 to 80.7% in genotype 3–3, the content of nicotinic acid appears to be less sensitive. It is relatively low (mean, 24.2 micrograms per gram of tissue) in the group of nine starchy types that

carried both *su*^{am} and *Du*, and higher by a factor of approximately 2 (mean, 50.5 micrograms per gram) in the group of seven types that lacked one or both of these alleles. The difference between the two means is highly significant, with a P value of less than 0.01. Within each of these two groups the variation among individual values is non-significant by the X^2 test; nevertheless, there is a marked tendency for nicotinic acid to reflect changes in genotype correlated with changes in starch content. This has been indicated in a column of subgroup means. Thus genotype 0–0 had a nicotinic acid content of 56.7 micrograms per gram of tissue; genotypes 0–1, 0–2 and 0–3 averaged 51.6 micrograms per gram; genotypes 1–0, 2–0 and 3–0 averaged 47.4, and so on. For the entire series, the correlation coefficient between starch and nicotinic acid is -0.97, with a P value of less than 0.01.

It has been shown that the apparent nicotinic acid content of cereal products is influenced by the method of extraction; thus wheat autoclaved with 0.1 N sulfuric acid assayed 37 micrograms of nicotinic acid per gram of tissue, but when treated with 1.5% sodium hydroxide or 3 N hydrochloric acid it assayed 72 to 75 micrograms per gram.[10] One N sulfuric acid also gives maximum nicotinic acid values in cereals,[11] and the data reported in table 1 were obtained by its use. To determine whether there might be differences among the genotypes in the proportion of nicotinic acid released by strong and mild hydrolyses, five of the genotypes were assayed after autoclaving with 0.1 N sulfuric acid. The results of these assays are presented in table 2 and compared with those from table 1. In each case the value obtained by mild hydrolysis was markedly lower than that obtained by strong hydrolysis. With the exception of genotype 0–3, the percentage of total nicotinic acid made available by this mild treatment was about the same for all, however, and this suggests that *su* and *du* exert no differential action in this respect. Without further tests it cannot be determined whether the one notably lower percentage (50.3, for genotype 0–3) has significance.

TABLE 2

EFFECT OF STRONG AND MILD HYDROLYSES ON THE APPARENT NICOTINIC ACID CONTENT OF FIVE MAIZE ENDOSPERM GENOTYPES

GENE DOSAGE, *su*^{am}*Du*	NICOTINIC ACID RELEASED BY		
	STRONG HYDROLYSIS, MICROGRAMS PER GRAM OF TISSUE	MILD HYDROLYSIS	
		MICROGRAMS PER GRAM OF TISSUE	PER CENT OF STRONG HYDROLYSIS
3–3	21.7	15.0	69.1
1–1	29.4	19.3	65.6
3–0	44.3	29.6	66.8
0–3	56.3	28.3	50.3
0–0	56.7	39.0	68.8

Results of exploratory assays for thiamin and biotin, carried out on the same five genotypes, are shown in table 3. These data must be considered only approximate, but they indicate that the two vitamins bear a relationship to the carbohydrate system similar to that of nicotinic acid, in that they are low in starchy types and higher in sugary ones. The biotin level found for genotype 3–3 agrees well with the value reported by Cheldelin and Williams[12] for white corn meal, but the thiamin level for this genotype is less than one-tenth as much as that reported by Van Lanen, Tanner and Pfeiffer[13] for a composite sample of yellow maize.

Discussion.—The data of Burkholder, McVeigh and Moyer,[1] together with those of Mather and Barton-Wright[3] and of the present paper, demonstrate a general correlation between sugary-type maize endosperm and relatively high nicotinic acid content. Three different gene combinations, *su^{am} du*, *su Du* and *su du*, when homozygous for their recessive members, are sugary type and high in nicotinic acid, while two combinations, *Su Du* and *su^{am} Du*, are starchy type and low in nicotinic acid. Thus

TABLE 3

THIAMIN AND BIOTIN CONTENT OF FIVE MAIZE ENDOSPERM GENOTYPES

GENE DOSAGE, *su^{am}Du*	MICROGRAMS PER GRAM OF TISSUE	
	THIAMIN	BIOTIN
3–3	0.18	0.07
1–1	0.37	0.09
3–0	0.42	0.14
0–3	0.98	0.13
0–0	0.88	0.15

changes involving either the *Su su^{am} su* series or the *Du du* pair are independently effective. Endosperm tissue from the gene combinatoin *Su du* has not been assayed, but since this is a starchy type it is probable that the nicotinic acid content would be low.

The present data, like those on the carbohydrates,[4] have been presented on a percentage basis, or its equivalent. In this genotypic series, however, about 1.37 sugary-type endosperms were required to equal the weight of 1 starchy-type. If the number of cells laid down in an endosperm were essentially the same in the two types (with the weight difference due primarily to differential amounts of stored carbohydrates), and if the production of the vitamins were a cell function unrelated to the genes in question, then sugary tissue might be expected to yield about 1.37 times as much as an equivalent weight of starchy tissue. Such a situation, however, does not account for the extent of the differences found. Studies on starchy maize hybrids[14] have shown that variation in nicotinic acid content can also occur as a function of the hybrid, and of the year and the location of the planting. In the present series these factors were not variables.

For comparable types, the nicotinic acid values reported here average higher than those of Mather and Barton-Wright;[3] they agree rather well with the starchy-type average of Burkholder, *et al.*,[1] but are higher than the sugary-type average reported by these workers. These differences are probably a function of the genetic backgrounds involved, for all these authors reported wide variation in nicotinic acid for both sugary and starchy kernels of various varieties. Their assays differed also in that they were apparently made upon whole kernels.

The mechanisms underlying the relationships between the carbohydrates and the three B vitamins are not known, and the possibilities are many. The primary action of the genes may relate either directly or indirectly to the carbohydrates or to any one or all of the vitamins.

It has been suggested[4] that in the starch-synthesizing processes certain steps relating to the action of phosphorylase are affected by *su* and *du*. If this is correct, changes in vitamin content may be secondary. This is plausible, since (*a*) vitamin content appears to be less sensitive than carbohydrate content to changes in genotype; and (*b*) the three vitamins all change in the same direction, as if in response to some general influence. The degree of starch accumulation might constitute such an influence. In starchy endosperms the cells become primarily storehouses for starch grains, and the production of other substances may be limited as a result.

In microorganisms shunt mechanisms exist,[15] which, although often entirely inoperative in the presence of low concentrations of (soluble) carbohydrate, form substantial quantities of certain metabolic products when carbohydrate is plentiful. In the present case the sugary maize types, in which soluble carbohydrates accumulate, may produce greater quantities of vitamins by such a mechanism. It is also possible that a portion of the vitamins or some of their components are used up (from an otherwise constant store) in the process of polysaccharide synthesis, so that smaller amounts remain in starchy kernels than in sugary ones. Thus nicotinamide, which is related to cozymase, may suffer loss due to "attrition" or other causes[16] in the course of this activity.

If it is the vitamins which the genes more directly control, different explanations are required. Conceivably, the greater quantities of vitamins in sugary genotypes could interfere with synthesis of starch or contribute to its breakdown. Again, in microorganisms it has been shown[17] that high vitamin levels can cause metabolic upsets.

It has been pointed out (by E. G. Anderson, in personal communication to the writers) that thiamin and perhaps precursors of other vitamins may appear in the endosperm by translocation from the leaves. This could imply control of these substances by genes in the diploid plant tissues, which would require a reconsideration of the gene dosages involved.

Data are accumulating which make possible the breeding of maize for

greater nutritive value. It has been known for some time that the content of vitamin A-active carotenoids is controlled by a gene pair, Y_1 and y_1; yellow maize contains these carotenoids in direct linear proportion to the doses of Y_1 present; white maize lacks them.[18] In studies on the inheritance of oil, inbreds of high and low oil content have been obtained, and genetic effects are detectable in their hybrids;[19] the inheritance of proteins and amino acids is likewise being studied. In sweet corn, genes such as *du*, in conjunction with the common su_1, provide possibilities for the development of improved eating quality. The low nicotinic acid content of starchy maize is well known, especially because of its apparent relation to pellagra. A somewhat higher content can be obtained by selection among starchy hybrids,[14] and for purposes for which sugary types can be utilized the still higher levels represent an additional advantage.

Summary.—In a series of relatively isogenic mature maize endosperm genotypes, inversely related changes in nicotinic acid and starch content were found to be conditioned by genes at both the su_1 locus and the *du* locus. Nicotinic acid content appeared somewhat less sensitive to changes in genotype than did starch content. On a percentage basis sugary types averaged about twice as high in nicotinic acid as starchy types.

The proportion of total nicotinic acid made available by mild hydrolysis was similar in all but one of a group of contrasting genotypes tested.

Exploratory assays for thiamin and biotin indicated that their content in the endoserm, like that of nicotinic acid, is low in starchy types and higher in sugary ones.

Possible mechanisms underlying these relationships are discussed.

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A GENE-CONTROLLED REACTION IN NEUROSPORA INVOLVING THE SYNTHESIS OF PANTOTHENIC ACID

BY ROBERT P. WAGNER AND BEVERLY M. GUIRARD

GENETICS LABORATORY AND THE BIOCHEMICAL INSTITUTE, THE UNIVERSITY OF TEXAS
AUSTIN, TEXAS

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The postulate that the gene is the ultimate agent controlling biochemical reactions is accepted by many geneticists as a working hypothesis. The evidence is based on the fact that gene changes bring about changes in biochemical reactions, and sometimes create a "genetic block" which makes it impossible for products to be formed. Furthermore, each gene is assumed to be specific for a specific type of biochemical reaction, with the corollary

logically following that there exists a one-to-one relationship between the gene and the enzyme.[1,2] The experimental evidence confirming this relationship is scanty, because its direct investigation is made difficult by inadequate information on known gene-controlled reactions, and the technical difficulties of carrying out many of these reactions *in vitro*.

That the ability to produce a specific enzyme is, in fact, inherited has been demonstrated in at least two cases. The clover, *Trifolium repens* has been shown to possess a genetically determined enzyme, linamerase, which hydrolyzes the glucosides, linamerin and lotaustralin.[3] Some rabbits possess an atropinesterase in their blood which hydrolyzes atropine and monoacetylmorphine, and the ability to produce this enzyme is incompletely dominant to the condition in which the enzyme is absent.[4] In both of these cases the enzyme activity can be demonstrated *in vitro*. The substrate and end-products have been chemically characterized, and the presence or absence of the enzyme shown to be an inherited trait.

It is the purpose of the present investigation to make a direct study of the gene-enzyme relationship in the fungus, Neurospora. Two strains of Neurospora obtained from Dr. G. W. Beadle at the California Institute of Technology were used, the Emerson Wild Type 5265A and Pantothenicless 5531A. The pantothenicless mutant is unable to grow unless supplied with pantothenic acid, a vitamin not required by the wild type in its medium. It has been reported that 5531 has the ability to synthesize both β-alamine and pantoyl lactone (*DL*-α-hydroxy-β,β-dimethyl-γ-butyrolactone), and therefore presumably has a genetic block for the reaction coupling these fragments to produce pantothenic acid.[5] This mutant differs from the wild type by a single gene, and the gene locus has been approximately located on one of the seven chromosomes of Neurospora.[6]

Experimental.—Both wild type and pantothenicless Neurospora were grown in basal medium[7] with 1 γ per ml. of calcium pantothenate in the pantothenicless cultures. Mycelial pads were harvested at the end of 72 hours, washed thoroughly with sterile, distilled water and transferred aseptically to sterile flasks containing 25 ml. of distilled water. To one set of flasks 5 ml. each of 0.1 *M* solutions of β-alanine and pantoyl lactone was added. The cultures were incubated at 28°C. with constant shaking. Flasks removed after different intervals of time were steamed for about ten minutes; the mycelium was harvested and dried at 90°C., and then weighed. The fluid contents of the flasks were assayed for pantothenic acid with *Lactobacillus arabinosus* according to the method of Skeggs and Wright.[8] One per cent glucose (instead of two per cent) was used in the assay medium and growth response was measured turbidimetrically after an incubation period of 17 to 18 hours instead of acidimetrically after 72 hours' incubation. The amount of pantothenic acid produced in the medium per mg. dry weight of mycelium was calculated. The data from a 41-

hour run are presented in table 1. They show that the wild type produces considerable amounts of pantothenate in the presence of β-alanine and pantoyl lactone, and very little in their absence, whereas the pantothenicless mutant produces none in either case. There can be little doubt that wild type Neurospora is capable of coupling the pantoyl lactone and β-alanine to form pantothenic acid, and that the pantothenicless mutant has a genetic block at this coupling reaction.

It should be noted, in connection with the experiment reported above, that no attempt was made to control the pH, but that the pH remained at about 6.0 during the course of the incubation. It was also found that toluene, which was used as a preservative in one experiment, completely inhibited the production of pantothenate by wild type.

In vitro experiments were carried out with a preparation made by growing large amounts of mycelium in minimal medium. Growth was per-

TABLE 1

PRODUCTION OF PANTOTHENATE BY INTACT, RESTING MYCELIUM OF WILD TYPE AND PANTOTHENICLESS *Neurospora* ("PRECURSORS" REFERS TO β-ALANINE AND PANTOYL LACTONE)

PERIOD OF INCUBATION (HOURS)	MICROGRAMS PANTOTHENATE PRODUCED PER MG. DRY WEIGHT MYCELIUM			
	WILD TYPE		PANTOTHENICLESS	
	PERCURSORS	NO PERCURSORS	PERCURSORS	NO PERCURSORS
0	0.01	0.01	0.01	0.00
5.5	0.09	0.01	0.01	0.01
16.5	0.35	0.04	0.01	0.03
22.5	1.23	0.11	0.03	0.03
28.5	2.50	0.11	0.02	0.03
41.0	5.00	0.06	0.02	0.02

mitted to proceed for 48 hours with aeration, and the mycelium then washed with distilled water and four successive portions of dry acetone. After being dried thoroughly the preparation was ground to a fine powder and stored in the refrigerator. The yield is about 20 to 23 g. of acetone-dried mycelium per 15 liters of culture fluid.

The effect of hydrogen-ion concentration on the production of pantothenate was tested by using 50 mg. of the dried mycelial preparation per flask suspended in 25 ml. of 0.04 *M* potassium phosphate buffer solution containing 1 ml. each of 0.1 *M* solutions of pantoyl lactone and β-alanine. The solutions were sterilized and the mycelial preparation added aseptically. The reaction mixtures were incubated at 25°C. and shaken constantly for 24 hours. They were then steamed, the solids separated by centrifugation and the centrifugates assayed for pantothenate. Since in alkaline solution there is a certain amount of synthesis of pantothenic acid from β-alanine and pantoyl lactone when these compounds are heated together in solution, a control series of flasks was set up which contained no mycelium. The contents of these flasks were assayed and the values obtained subtracted

from the pantothenate values found in the flasks with mycelium. The results expressed in micrograms pantothenate per mg. acetone-dried preparation are given in table 2. They show that the optimal pH range under the conditions of this experiment lies above pH 6.0.

TABLE 2

THE EFFECT OF HYDROGEN-ION CONCENTRATION ON THE SYNTHESIS OF PANTOTHENATE BY THE ACETONE-DRIED MYCELIUM OF WILD TYPE *Neurospora*

pH	MICROGRAMS PANTOTHENATE PRODUCED PER MG. MYCELIUM
5.0	0.01
5.5	0.29
6.0	0.70
6.5	0.79
7.0	0.59
7.5	0.62
8.0	0.59
9.0	0.54

The effect of omitting the precursors, β-alanine and pantoyl lactone, is shown in table 3. In this experiment incubation time was 24 hours at 25°C. The blank contained only phosphate buffer at pH 6.0 and the mycelium. The data demonstrate the dependence of the reaction on the presence of both of the precursors.

TABLE 3

THE EFFECT OF THE PRESENCE OF β-ALANINE AND PANTOYL LACTONE ON THE PRODUCTION OF PANTOTHENATE BY WILD TYPE *Neurospora*

	MICROGRAMS PANTOTHENATE PRODUCED PER MG. MYCELIUM
Pantoyl lactone + β-alanine	0.58
Pantoyl lactone	0.13
β-alanine	0.11
Blank	0.10

Table 4 shows the effect of increasing the concentration of the mycelial preparation on the amount of pantothenate synthesized during an incubation period of 24 hours at 25°C. The total amount of pantothenate produced per flask is given in the table.

The release of pantothenic acid preformed in the wild type mycelium used in the preceding experiments might be expected to have some effect on the results. For this reason intact and acetone-treated mycelia were assayed for their content of the vitamin. Preparations from fresh cultures were homogenized in acetate buffer at pH 4.5 and treated with Clarase and Caroid enzyme mixtures for 24 hours at 37°C.[9] No pantothenate was detected by assay in the case of the intact mycelium, probably because of incomplete digestion. However, the acetone-treated mycelium contained 0.014 γ pantothenate per mg., a concentration very close to the pantothen-

ate content reported for *Oidium lactis*.[10] If we accept this value as a reasonably accurate one for wild type Neurospora then it becomes quite evident that pantothenate must actually have been synthesized, and not merely released after having been formed during the actively growing phase of the mycelium.

Discussion.—The data presented demonstrate that there is an enzyme system present in wild type Neurospora which catalyzes the synthesis of pantothenic acid from β-alanine and pantoyl lactone. This reaction can be demonstrated both *in vivo* and *in vitro*, although the rate is not as great in the latter case. Efforts made to increase the activity of the acetone-dried preparation to at least that of the intact mycelium have not met with consistent results. At present it appears that glucose and coenzymes such as adenosine triphosphate, pyridoxal phosphate and Coenzyme I may stimulate the synthesis of pantothenate *in vitro*, but the proper conditions have not yet been worked out.

TABLE 4

THE EFFECT OF CONCENTRATION OF THE MYCELIAL PREPARATION ON THE SYNTHESIS OF PANTOTHENATE

MG. MYCELIUM PER FLASK	MICROGRAMS PANTOTHENATE PRODUCED PER FLASK
25	37
50	55
75	93
100	175

The present investigation indicates that the reaction leading to the production of pantothenate from β-alanine and pantoyl lactone in Neurospora should be useful in studying directly the gene-enzyme relationship in this organism.

The authors gratefully acknowledge the interest shown in their work by Dr. H. K. Mitchell of the California Institute of Technology whose suggestion concerning the possible usefulness of the pantothenicless mutant of Neurospora provided the initial stimulus for this investigation.

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MULTIPLICATIVE SYSTEMS, I

BY C. J. EVERETT AND S. ULAM

LOS ALAMOS SCIENTIFIC LABORATORY

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1. Introduction.—The purpose of this note is to introduce a formalism suitable for the description of systems which consist of particles of different types, each particle transmuting with given probability into a group of particles of these types, the number of particles in the system varying with time.[1, 2, 3] The type of a particle may refer to characteristics like position or momentum as well as to intrinsic properties.

The phase space in which we are interested here is that of all possible futures or genealogies of such systems, each such genealogy being considered as a point of the space. Given the elementary probabilities of transmutation, one can introduce a notion of probability or measure for certain subsets of the phase space.

The formalism is general enough to include as special cases the multiplication of bacteria, radioactive decay, cosmic ray showers, diffusion theory and the theory of trajectories in mechanical systems.

Detailed discussion of specific cases, at present in the form of reports, will be published elsewhere.

2. Remark on Measure.—It is convenient to have at hand some simple axioms on which the classical measure theory may be shown to rest. Suppose $\Gamma = \{\gamma\}$ is a point set, $I = \{i\}$ a class of distinguished subsets i of Γ called intervals, including the empty set θ and the entire set Γ. Denote by J the class of all subsets $S = \Sigma i$ which are sums of a finite or countable class of intervals (all sums hereafter are supposed to be over a finite or countable number of summands). Suppose that intervals satisfy the axioms:

I.1. Every set S of J can be represented as a sum of mutually disjoint intervals.

I.2. The complement $i' = \Gamma - i$ of an interval i is in J.

I.3. The set product ij of two intervals i, j is an interval.

Assume further

M.1. To every interval i is assigned a non-negative real number $m(i)$ called its measure.

M.2. $m(\Gamma) = 1$.

M.3. If $i = \Sigma i_\mu$, where the i, i_μ are intervals and the i_μ are pairwise disjoint, then $m(i) = \Sigma m(i_\mu)$.

An additive class C of subsets of Γ is one such that

C.1. All intervals belong to C.

C.2. If $A_1, A_2, \ldots$ are in C, so is ΣA_μ.

C.3. If A is in C, so is A'.

The intersection of all additive classes consists of the Borel sets. Thus all Borel sets are contained in any additive class.

If one defines outer measure $O(U) = glb\{m(S);\ U \subset S \epsilon J\}$ and inner measure $I(U) = 1 - O(U')$ as usual for an arbitrary subset U of Γ, one can show on the basis of the stated axioms that the class of all measurable sets M (for which $I(M) = O(M)$) is an additive class. Moreover, defining measure $m(M)$ for measurable sets to be the common value $I(M) = O(M)$, one can show $m(M) \geqq 0$; $m(\Sigma M) = \Sigma m(M)$, for disjoint measurable sets M; and if M is an interval, the assigned measure coincides with the original interval measure.

3. The set Γ_t of Graphs.—Consider t types of particles such that a particle of type i may produce, upon tranformation, $j_1 + \ldots + j_t \geqq 0$ of such particles, of which j_ν are of type ν, $\nu = 1, \ldots, t$. We suppose transformation times are the same for each type and hence that generations may be counted unambiguously. We agree to consider zero generation as consisting of one particle of a fixed type i. Then we consider the set Γ_t of all possible infinite histories or genealogies of such a particle, that is, the infinite records of the transformations of this particle and all its progeny through all generations $k = 0, 1, 2, \ldots$.

We may represent a genealogy in the plane if we make the following conventions:

(*a*) t different "colors" are assigned to the t types, a particle of type ν in the kth generation being represented by a dot of appropriate color.

(*b*) If a particle of type μ in generation k is transformed into no particle, i.e., if it dies or escapes, this is indicated by a sequence of Δ's (Δ = death) vertically below it, one in each succeeding generation.

(*c*) If a particle of type μ in generation k is transformed into $j_1 + \ldots + j_t > 0$ particles, j_ν of type ν, this is indicated by a branching from the corresponding dot of color μ in the kth generation to $j_1 + \ldots + j_t$ dots, j_ν of color ν, in the $k + 1$st generation, dots of the same color being placed consecutively and colors ranging in order $\nu = 1, \ldots, t$ from left to right.

Thus we may regard the set Γ_t of all such graphs γ in the plane. If γ is a graph, γ_n denotes the upper segment of γ from generation 0 through generation n. If $\gamma = \bar{\gamma}$, then $\gamma_n = \bar{\gamma}_n$ for all n. If $\gamma \neq \bar{\gamma}$, define $k(\gamma, \bar{\gamma})$ as the least integer n for which $\gamma_n \neq \bar{\gamma}_n$.

The set Γ_t has the natural metric: $d(\gamma, \bar{\gamma}) = 0$ for $\gamma = \bar{\gamma}$, $d(\gamma, \bar{\gamma}) = 1/k(\gamma, \bar{\gamma})$ for $\gamma \neq \bar{\gamma}$. The metric space Γ_t resulting is 0-dimensional, separable, and complete, but *not* locally compact.

A graph γ is said to terminate in case it contains no particles (only Δ's) in some generation. The set T of all terminating graphs may be split into the disjoint summands $T_0, T_1, \ldots$, where T_n denotes the set of all

terminating graphs $\tau^{(n)}$ which contain at least one particle in generation n, but none in the next.

By an interval of order n is meant the set $i(\tau^{(n)})$ of all graphs γ such that $\gamma_n = \tau_n^{(n)}$ where $\tau^{(n)}$ is a particular graph in T_n. The only interval of order 0 is Γ_i itself. We now define *interval* to be either θ, or any single graph γ, terminating or not, or any interval of order n, $n = 0, 1, \ldots$. It is easy to see that axioms I.1, 2, 3 are satisfied. Indeed we have the simple property that the intersection ij of two intervals is either θ or i or j.

4. *Measure in the Space of Graphs.*—While the notions of graph, distance and interval are geometric in character and depend only on the number t of types, measure may be introduced in various ways. One of the simplest, but by no means the only one to which the theory has been applied, may be imposed as follows. Suppose we assign a probability $p(i;\ j_1, \ldots, j_t)$ to the event that a particle of type i should produce, upon transformation, $j_1 + \ldots + j_t$ particles, j_ν of type ν. Then every segment γ_n of a graph γ has an associated probability $p(\gamma_n)$ that the event described by γ_n should occur. If we assign to intervals a measure by $m(\theta) = 0$ $m(\tau^{(n)}) = p(\tau_{n+1}^{(n)})$, $m(i(\tau^{(n)})) = p(\tau_n^{(n)})$, and $m(\gamma) = \lim p(\gamma_n)$ for γ non-terminating *it turns out that M.1, 2, 3 are satisfied* and thus Borel sets based on our intervals are measurable in the sense of §2. (Non-compactness of intervals makes M.3 non-trivial.)

The procedure applies in much more general systems where the transition probabilities are functions of the time.

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ON THE ZEROS OF THE DERIVATIVE OF AN ENTIRE FUNCTION OF FINITE GENRE

BY MORRIS MARDEN

DEPARTMENT OF MATHEMATICS, UNIVERSITY OF WISCONSIN, MILWAUKEE

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Let $E(z)$ denote an entire function of genre p:

$$E(z) = e^{P(z)} z^r \Pi_{j=1}^{\infty} [1 - (z/z_j)] \exp \textstyle\sum_{k=1}^{q} [(z/z_j)^k/k],$$

where $P(z)$ is a polynomial of degree p_1 and $p = \max(p_1, q)$. Clearly, the zeros of its derivative $E'(z)$ depend not only upon the zeros z_j of $E(z)$ but

also upon the polynomial $P(z)$. This contrasts with the fact that the zeros of the derivative of a polynomial depend only upon the zeros of the polynomial. It accounts for the difficulty experienced by some previous investigators who, seeking to extend to entire functions the well-known results on the zeros of the derivative of a polynomial, found it necessary to assume $P(z) \equiv 0$ or to restrict p to the values 0 or 1.[1]

In the present note, the difficulty due to the $P(z)$ is circumvented by establishing for $E'(z)$ the following new representation.

THEOREM 1. *Let $E(z)$ be an entire function of genre p. Let $z_0 = 0$, $z_1, z_2, \ldots$ denote the zeros of $E(z)$ with the multiplicities $m_0, m_1, m_2, \ldots$, respectively, and let $Z_1, Z_2, \ldots, Z_p$ denote any p zeros of $E'(z)$. Then*

$$E'(z) = E(z)\sum_{j=0}^{\infty}\{[m_j/(z - z_j)]\Pi_{k=1}^{p}[(Z_k - z)/(Z_k - z_j)]\}.$$

Theorem 1 may be proved by extending to meromorphic functions our recent results[2] on the zeros of rational functions of the form

$$F(z) = \sum_{j=0}^{p-1} A_j z^j + \sum_{j=1}^{n} m_j/(z - z_j),$$

where the A_j are arbitrary complex constants and the m_j are points in a convex sector. It may also be proved directly by eliminating $P(z)$ from $E'(z)$ by use of the equations $E'(Z_k) = 0$, $k = 1, 2, \ldots, p$.

An immediate consequence of Theorem 1 is

THEOREM 2. *If $Z_1, Z_2, \ldots, Z_{p+1}$ are any $p + 1$ zeros of the function $E'(z)$, then*

$$\sum_{j=0}^{\infty} m_j/[(Z_1 - z_j)(Z_2 - z_j)\ldots(Z_{p+1} - z_j)] = 0. \qquad (1)$$

The study of the argument of each term in equation (1) leads now to

THEOREM 3. *Let K denote the smallest convex infinite region which encloses all the zeros of $E(z)$ and let $S(K, \psi)$ denote the star-shaped region comprised of all points from which K subtends an angle of at least $\psi = \pi/(p + 1)$. Then at most p zeros of $E'(z)$ lie outside of $S(K, \psi)$.*

When $p = 0$, the region $S(K, \psi)$ coincides with K. Thus Theorem 3 is seen to be a generalization of the Lucas Theorem that any convex region enclosing all the zeros of a polynomial also encloses all the zeros of its derivative.

In particular, if K is chosen as a convex sector, the following result is obtained.

THEOREM 4. *Let α and β be non-negative numbers such that $\alpha \leq \beta$, $\alpha + \beta = \pi/(p + 1)$. If all the zeros of $E(z)$ lie in the sector $|\arg z| \leq \alpha$, then at most p zeros of $E'(z)$ lie in the sector $|\arg(-z)| \leq \beta$.*

Equation (1) also leads to an elementary proof of the following result of Laguerre and Borel.[3]

THEOREM 5. *Let $E(z)$ be a real entire function with only real zeros. Then*

$E'(z)/E(z)$ has at most p real and non-real zeros in excess of a single zero between each pair of successive zeros of $E(z)$.

All of the above theorems remain valid if $E'(z)$ is replaced by $H(z) = E'(z) + Q(z)E(z)$ where $Q(z)$ is an arbitrary (real, in the case of Theorem 5) polynomial of degree not exceeding $p - 1$. For, the function $E_1(z) = E(z) \exp. \int Q(z)dz$ is an entire function of genre p possessing the same zeros as $E(z)$ and its derivative $E_1'(z) = H(z) \exp. \int Q(z)dz$.

A more detailed account of the above results, together with additional applications, will be published elsewhere at a later date.

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TWENTY EXACT FACTORIALS BETWEEN 304! AND 401!

BY HORACE S. UHLER

YALE UNIVERSITY

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In the year 1944 the author published privately a little book entitled *Exact Values of the First 200 Factorials*. Subsequently he computed with great care the exact values of $n!$ from $n = 201$ to $n = 300$. The data of this third century have not appeared in print. One consultable copy has been deposited in the library of Brown University, Providence 12, Rhode Island, and another copy is in the possession of Doctor J. C. P. Miller, Technical Director of Scientific Computing Service Limited, 23 Bedford Square, London, W.C. 1, England.

Recently the author has computed a skeleton table of 42 exact factorials beginning with 303! and ending with 400!. This table was built up by first calculating the values of $n!$ for which $n + 1$ was one of the 17 primes from $n = 307$ to $n = 401$, so that Wilson's theorem could be applied as a more exacting check in addition to congruence testing with moduli such as $10^5 + 1$, $10^3 + 1$, etc. Incidentally the values of 350!, 372!, 375!, 378! and 400! as found by the author in February, 1945, were reproduced identically in the work performed three years later. In order to make a few of these arithmetical constants available to other investigators requiring exact values in the fourth century of $n!$ the following table of equally spaced but non-consecutive data is presented.

$305!/10^{75} =$

78
16143 67270 06180 26842 21781 89219 57830 70329 83534 63749 47728
66413 96628 16717 92393 86921 72994 33010 41362 09502 42202 06171
62425 70005 87970 32565 74046 67837 53084 59719 20375 86704 96780
27329 65215 01067 57207 75248 99979 71751 10374 28293 65958 21786
93340 33476 64249 90189 04484 54625 12887 81096 82388 28344 65942
17304 84960 08989 52299 90223 10631 53132 49820 70510 36643 66235
87970 71920 65590 58389 70553 59515 39835 59467 74139 56369 78246
12784 47724 93307 09006 35589 61199 74856 43598 64728 70357 22731
42776 53698 93630 46708 22644 31683 43792 23082 92544 25088 11617
53040 88347 71608 33635 71475 56100 82949 78164 20479 73459 23072

$310!/10^{76} =$

2166 32307 39615
57382 46323 52647 78187 95476 79518 99579 52188 49117 49141 81516
69177 68056 63364 68240 67318 71222 58715 50633 17192 53555 64906
02511 56380 44857 87856 98918 43414 84630 74133 24707 51131 32472
66239 23677 47417 72624 82291 59184 42537 62262 89172 60707 31456
57018 79241 49987 04985 83771 09316 72970 22939 23879 45276 93358
86726 60161 75252 63987 11567 16646 56686 20269 46552 69368 82261
63413 89016 78640 80356 86331 68719 62066 19987 30510 47909 31513
70148 32280 60402 28659 87648 08466 70375 93175 75294 06975 27745
32860 60968 62580 63385 63798 61981 33889 16877 69791 67062 98763
90059 94763 57218 32760 86222 26812 05438 36586 57430 21171 01568

$315!/10^{77} =$

65076 28432 35846 76142 84900
91280 84648 67246 93577 25980 02786 74197 38453 71750 72340 30305
08722 14393 27856 51973 27268 67012 22354 17592 68574 72307 60553
61061 94568 15248 53610 41430 89351 01368 99668 13922 65197 62140
64139 40839 25901 58615 43169 46059 44417 78372 98555 22524 09073
81753 22134 92158 19852 24245 53131 19258 22462 95412 09823 69427
31544 79121 68349 69260 74271 29127 63153 61009 53702 68216 65849
57188 45236 83646 81102 37278 43235 51317 63211 48729 06486 79100
13746 83046 89399 57977 04211 22719 41248 51953 75150 99839 36963
32916 48221 19039 16368 62309 74000 23742 66148 22088 25999 10187
80899 51827 27950 77930 26927 27664 80616 94761 43746 36373 93408

$320!/10^{78} =$

21 16103 34721 92524 82955 71704 10776 29865
87946 39108 37613 06765 57783 01557 80903 30844 47216 78617 88371
08317 09407 22591 24180 71083 82859 29587 26413 48645 16639 12600
40395 58390 89865 02774 34785 61543 14632 61485 73930 87562 33136
98969 64916 31377 72782 92965 20278 06263 04839 72525 43230 83321
24593 59203 45445 76046 93157 16688 80818 13860 83935 73770 52843
53395 86952 08617 42156 12749 63850 90743 60230 90498 20934 91713
47554 61873 01294 57049 38955 13272 46630 75880 43699 59040 93654
70934 95526 56965 61054 65403 72048 42102 66089 25808 49397 81640
19986 59344 25649 05462 74566 94123 26023 29181 22696 08558 33215
77599 89142 54964 92653 59278 84808 48689 20655 69846 12424 25344

$325!/10^{80} =$

74 39241 74558 20825 43735 34494 22105 61663 83967 17520
38059 07317 38414 66417 00760 47025 41857 66473 78675 82293 78970
54750 30667 62898 76155 84867 98163 29339 93348 42298 37744 03857
93161 46480 42003 51689 40232 91722 46643 76150 36938 24510 20933
16423 02926 22957 07373 81260 20152 52321 88823 20676 72634 49792
57687 87656 32541 67677 40192 65463 74562 08691 56586 99269 82630
01190 29558 78973 52044 43614 38588 21524 87400 48531 73763 00573
22078 77271 50660 94791 24466 15706 93170 75740 94166 74152 78143
81490 07929 38952 08733 37686 10203 73826 49092 43130 35525 30809
37831 71472 69624 77638 86024 68739 41043 51267 64486 39822 27540
74862 41909 29892 71284 09589 30646 07043 81537 21506 68598 96832

$330!/10^{81} =$

										2824
08462	25838	71086	56010	65535	09844	21358	23061	55484	47917	12597
38190	61204	08795	34106	75131	67173	45648	85871	83699	48575	84240
51605	24086	04307	34116	50670	52618	23551	53355	92584	91538	01248
88501	44424	17647	15270	10285	80624	04841	10526	90725	30716	90361
23418	80567	81083	95522	57654	49360	11494	92226	04307	54462	86770
76900	02535	50746	72950	34249	73082	81617	04937	16827	30704	92544
57005	43334	27097	51252	77956	18877	50731	81736	31303	45796	96036
35006	71701	03798	94122	54276	57240	69914	28638	65586	03964	57246
35073	71484	51362	95430	04002	37985	66203	76408	67840	59147	34449
85051	11170	69154	59551	70513	50965	81824	24889	07209	26775	93945
62763	90199	87779	97874	88537	30433	82846	84150	35962	15208	50944

$335!/10^{82} =$

							1	15632	33374	76057
93897	53538	04080	10912	68491	24676	06380	60093	13907	45734	40409
72220	62840	52553	61624	80175	28415	75881	72507	37601	10680	52800
94872	50352	03705	25604	15910	40543	98239	92655	53881	48596	22741
10477	33655	22467	39523	54575	91597	72316	07882	10176	61386	54718
92395	28832	63254	05333	46598	01513	83359	87995	14906	97445	91206
79882	41487	93034	03739	47786	88438	44582	06602	87501	19045	25452
34348	98810	30684	78921	64591	78597	76099	81759	32112	25628	97869
32411	05858	98441	58812	31372	54159	30812	86923	47611	05015	13791
81839	45036	48165	36811	00870	74766	75969	31540	60988	93507	74705
20248	94533	26775	83896	60107	30186	90638	57812	71218	27780	08281
48363	62059	19067	07450	13954	58751	46455	28828	73065	19947	50976

$340!/10^{83} =$

						51	00864	47210	37110	80930	19328
39272	93363	03446	71982	88537	60394	68582	43798	11307	08498	58229
21313	63729	54649	89902	03510	11761	05977	88061	33788	93649	35774
34500	36168	34151	88982	47240	13463	38884	86114	14227	40713	13888
05908	33811	29369	42713	65431	21020	13237	23370	85743	88954	02320
41192	95061	83969	59886	33829	52818	38936	92358	32500	91164	12814
17808	59964	38696	88726	98869	90263	12600	54113	91824	90611	59323
94760	45067	55237	53157	38712	39151	79975	20501	62388	22164	26473
86346	85875	38818	06087	52182	40504	10520	57069	47492	36518	81064
25431	86264	10104	74387	42041	85541	64289	64941	04086	29854	29878
77862	04180	85044	53671	65509	33980	52608	36237	88608	80585	11128
06177	13156	22325	53426	32769	29061	01835	27477	35327	20140	12416

$345!/10^{84} =$

				2421	56386	50792	34655	87000	53691	98585	55701
20556	04025	86527	34839	78326	70399	61720	17832	35931	74739	04791
36170	79695	53150	26894	73012	21382	08891	34885	85399	28184	38056
44508	02014	82863	67524	04948	02269	82311	01258	81000	28468	73771
04376	40079	22001	65127	85590	84980	47507	34795	54466	03093	96432
69870	87311	39427	46842	37308	39850	29113	04969	71971	50980	68025
49750	49007	30580	21701	65732	70011	69846	73789	24291	55078	08736
05154	73687	95426	02554	63555	84282	65690	30209	13423	59471	86350
86275	16511	20347	83535	42187	15104	58382	67239	16892	87475	25890
55970	84876	55213	48872	75308	84968	55871	63850	00436	98912	94795
27833	01034	05177	60688	34536	87157	29020	01533	68625	34353	87691
48712	01776	69920	58786	62858	55585	72655	44230	99917	84492	56448

$350!/10^{86} =$

12358 74058 26548 87501 43951 99766 54645 72245 32073 94691
95158 79429 33023 00930 35357 49131 42169 34583 29501 11784 45941
55210 94327 61532 44976 77618 92237 04344 49422 13964 09009 16694
90545 66125 51113 34533 06982 54556 07852 78983 64515 85122 90209
96499 77304 22679 48748 40601 81101 77641 37584 86813 75049 75397
32592 58825 41777 11770 66194 90238 36340 92545 89994 07933 46268
93194 60801 68889 86949 68499 43334 59029 36521 45557 84862 35393
91025 67266 74571 28468 24819 00414 60641 84543 88812 35334 64975
62117 92870 75018 58648 13576 43313 07515 33590 02713 29461 16326
14208 13403 66501 16689 05258 55733 50955 36024 61704 51786 97235
13653 70405 72203 62943 85680 47828 72788 27977 51141 19090 71460
91480 76811 31728 23218 29915 17416 47048 31579 98067 48729 01632

$355!/10^{87} =$

6 77376
79776 67418 36099 91145 74273 79142 17542 22965 94923 96674 85269
67948 42281 92577 25475 68718 76618 99834 45933 83880 00852 20067
66859 41701 64400 46981 51667 73291 99631 37983 30499 20048 47652
69211 33921 98225 85634 71407 45950 89179 92648 79045 82804 53482
95329 34402 31039 32710 67286 29446 84060 92412 17643 10350 09354
15071 36870 09762 70611 86515 42496 65836 80466 48264 70890 83152
52700 86372 02398 72164 48607 98664 82079 83395 29533 76062 21827
31586 53268 75385 27004 06698 59604 94844 22827 62427 33819 36514
84358 18140 02625 83842 48233 15155 34787 84690 78490 00319 93652
86972 90882 53919 73620 37005 91070 19241 32274 07960 77548 20804
63229 42413 38087 58738 22495 80491 18413 82129 64553 75338 50288
82630 03835 65229 48801 11161 99399 03669 44277 57585 80366 70464

$360!/10^{88} =$

398 31669 22118 81067
82059 90336 56471 84342 24120 60566 41437 01183 60876 81419 05250
78778 28777 19783 67867 90614 84962 36508 15370 93035 92350 12970
45164 32578 31407 44700 98769 49575 32389 15840 08581 16442 93357
64558 98753 75992 53484 56032 60865 02781 50327 11018 08348 16760
59630 30972 86523 60528 04284 29436 10644 52529 81359 91037 79308
18774 85511 24524 74332 24987 34122 59136 13236 87319 17415 05398
32960 42291 39669 14391 50433 27960 03064 68690 30670 76824 89567
38775 70873 54859 60504 25633 44537 36488 02120 53587 26905 56957
48062 64667 48658 11939 84051 00909 71364 83506 52527 17494 84080
03573 38638 70881 76356 70004 57650 97378 45399 27240 20434 42156
82704 41950 38802 61322 96257 83142 49284 57061 97046 20700 40415
51342 09534 71940 89863 85968 92833 35973 44225 59404 29503 52896

$365!/10^{89} =$

25104 12867 55587 32292 92944 37488
12027 70516 55202 69876 07976 68725 95193 90110 61382 20937 41966
60180 09000 25416 93761 72314 36098 23286 60708 07112 33699 79853
44536 79106 53872 38359 97043 55532 74093 76780 91491 42944 08643
16046 92507 45101 34847 02554 60140 98005 90796 55410 41195 49610
53118 86173 37343 51455 17193 28276 08477 55882 29169 02135 39123
47918 62747 01519 39680 85049 40722 60703 30012 46328 39880 05504
87427 99987 66904 16973 43786 10781 85344 66796 68715 11049 65388
81301 36836 19901 05291 80056 12584 45494 88648 61768 29158 26347
56414 89909 84138 06780 99996 04687 48814 67348 37340 69935 98387
91124 99595 75845 38873 61666 15330 93253 55125 68450 56046 38873
81297 02951 38115 18614 13688 92298 65100 05440 94394 30146 99244
11255 57552 79140 76049 27642 53740 25041 03910 56421 97900 32896

$370!/10^{90} =$

16 94213 78414 97677 14980 34360 30075 05751 79884
98526 65620 28567 19083 85739 75834 14630 44271 28457 69099 10556
45734 38389 63692 07629 59736 20538 52992 49859 15172 53291 16045
50605 24925 12753 30648 39291 55304 30725 44244 10170 09622 13277
09589 01110 21672 66208 37640 86326 80159 57769 37938 31169 49085
43218 54848 49734 38746 43632 33052 95793 30285 23312 39633 71425
93072 08890 73797 30245 28315 33903 67363 61090 94054 89619 62687
25563 17835 69154 28332 14179 68564 19238 71762 37187 65861 86085
92976 50059 48941 77965 90930 14447 02879 34628 00869 38422 66265
97744 04705 76318 83892 17541 82866 40801 14234 62726 22278 91895
20636 70305 45494 54203 49190 38529 63458 96040 54050 49820 43983
29354 26972 35900 42264 39512 45608 55397 99212 39031 45458 28703
98306 38826 37414 73701 87610 64076 40553 65686 99003 71078 67648

$375!/10^{93} =$

12 23197 99935 77775 43039 69618 24144 35162 61034 26617 76910
64536 25695 77925 77806 06759 41939 92280 46573 18461 01687 26782
25710 03107 94190 68074 39962 29568 36014 52548 06731 60842 01910
45075 25971 40941 19483 63857 80515 18320 89524 58185 11336 63561
23602 82539 94656 12094 59748 20721 31334 17742 46378 34418 90092
84130 44918 22114 02760 68740 11239 68686 25321 07007 62476 70111
36150 01670 11391 80232 70088 04755 53111 90619 92226 05070 32665
60109 19117 74798 82909 32496 49834 50739 53856 35116 54387 25712
59963 95422 98301 58661 85582 58435 62114 04943 50085 91617 82334
29034 09063 68839 74842 74664 42123 43086 33728 07958 39810 61102
13513 29535 61148 81688 64444 48735 72596 82637 63093 53951 75676
92235 76659 26676 72909 77647 15873 71794 82824 68011 07939 82003
05658 02166 43741 06889 75365 67417 77488 54283 88049 65564 08832

$380!/10^{94} =$

943 93168
35166 58867 93554 22817 58299 37281 47585 04032 64861 53629 48674
40001 07042 45127 34225 96890 47843 36560 50455 91347 30512 88470
75730 02822 59578 82791 11896 52265 92961 83926 70347 76856 57760
56456 17888 00392 69308 34123 98664 31499 07809 85234 90078 50619
01304 07985 25252 56361 35193 78619 88290 90641 81536 23632 16117
88837 69938 62150 25314 15986 81319 25331 98006 26643 01149 51917
98466 10485 92587 82855 56858 32339 61379 27770 97590 40186 50227
34476 03796 54358 27575 69195 31413 89397 53825 48193 35594 77692
29240 61513 44190 06943 60283 76253 31360 91446 21004 49102 41971
05636 06579 58334 60770 92618 33655 68594 53667 19124 82069 97050
21756 00464 15900 27865 01236 44293 05991 91665 93324 44813 26462
29613 54191 86241 84153 20160 21543 17437 56899 04305 31490 17526
76716 52093 95529 48051 22901 49804 42638 97333 46250 00976 54784

$385!/10^{95} =$

77789 25820 00226 82857
26252 46114 15309 14984 70558 30033 03410 98826 89650 25219 49696
79085 09462 50041 60614 77714 28400 32749 70096 00180 16880 25715
06007 60973 38706 36254 65151 97397 79798 79777 23879 66375 12249
85963 69895 63488 39607 93900 53459 32156 76317 53747 63295 92543
54510 91172 33552 74009 75225 03021 67318 74738 07397 90814 32227
88674 48117 85875 77495 62590 48468 26489 29702 78702 22627 46051
02072 86421 81787 64018 22851 01009 79403 76433 86965 05089 27547
88419 21622 59601 87559 78614 76842 62118 67938 80884 77835 52373
22973 15903 45851 74157 43852 45890 02638 31761 20681 01579 84267
06181 42271 52746 58208 74530 48037 97654 91556 99326 58041 83489
47419 61323 60265 36061 94370 32037 02535 56826 46278 40497 84483
05384 86783 77919 14952 61331 72785 58824 65027 08157 48037 12043
31197 62669 48667 91618 77759 74266 35292 58702 75040 92581 72416

$390!/10^{96} =$

				68	40121	77738	22066	23906	18964	87020
49476	31481	32747	70285	35066	11179	20282	47435	90161	45630	25958
15813	55764	00709	57506	24122	30994	80621	16147	86597	15395	88944
69691	32612	29689	90881	16302	04905	97055	74128	29408	11292	44511
37690	74225	18614	81190	22832	22676	38959	40834	09538	16716	84736
90144	16657	12949	47309	19174	48139	32033	97102	62195	66770	51353
41665	36533	05575	23282	74776	27056	92163	10894	64050	24495	34064
52641	73808	10191	99624	89292	31724	79753	76876	73260	90076	09292
52617	27008	63082	87559	48519	52005	44928	91329	28666	83679	53139
37443	76458	15544	65013	44573	00695	13994	81916	58099	62058	28255
45136	32941	27280	44585	03088	65616	07954	73749	38828	02304	54827
59566	42726	25262	00294	39437	36441	24020	21295	20450	86447	84395
62773	47809	03782	04531	07276	29068	39469	39866	22008	75524	55757
36315	55379	02670	45812	69487	01877	04539	11393	43943	14467	57376

$395!/10^{97} =$

		6412	28070	65834	24106	22415	93125	16580	47304	96357
87024	22053	71607	55807	20126	88489	56708	64509	19701	90015	64863
02485	38560	35488	52691	82750	76913	18517	38630	06440	24491	10408
65322	19290	59920	46283	83584	01757	94698	03393	34596	25781	14094
30070	64560	34908	78194	94315	43117	81988	26927	29553	14076	41555
09082	52633	11267	50827	75952	38905	10563	63700	31748	89013	91022
38531	73584	82407	31077	73540	81436	19537	08102	21335	57278	28043
65810	54163	28037	51254	21717	90143	16721	93988	19061	80759	26665
52578	97035	66708	64409	01654	02267	38728	56106	88950	88806	56182
15002	12091	79017	73406	43833	73487	87366	01446	24545	25826	37274
58417	47046	70552	84850	52979	65994	71285	27727	83551	08789	02120
08596	77481	59110	03145	42365	21007	01698	79736	04888	97935	18640
21700	90452	10565	26818	12901	25372	41430	45005	60198	08312	84886
81894	23869	06626	37443	78647	94187	84777	19873	44441	93099	28448

$400!/10^{99} =$

64034	52284	66238	95262	34797	03195	03005	85070	25830	26002	95945
86844	45942	80239	71691	86831	43627	84786	47463	26467	62943	50575
03585	68108	48298	16288	35174	35228	96198	86468	02997	93734	16541
50838	16242	64619	42352	30704	62443	25015	71444	86708	90662	77391
49181	17331	95599	64407	09549	67134	52904	77020	32243	49112	10797
59328	07951	01545	37266	72516	27877	89000	93497	63765	71032	63503
31533	96534	98683	86831	33935	20243	73788	15778	67915	06311	85870
26182	70169	81974	00629	83025	30859	12983	46162	27230	45583	39520
75961	15053	02236	08681	04332	97255	19485	26744	32232	43866	99484
22404	23259	98055	51610	63594	23769	61399	23191	71340	63858	99653
79701	47827	20660	63202	17379	47201	03213	56624	61380	90779	42304
59736	06995	67595	83609	61587	15129	91382	22865	78579	54936	16176
54480	45322	20078	25818	40084	84364	15591	22945	42753	84803	55837
45180	22675	90006	13995	60145	59520	61272	11192	91810	50324	91008

THE DETERMINATION OF HEREDITARY ANTIGENIC DIFFERENCES IN GENICALLY IDENTICAL PARAMECIUM CELLS*

BY T. M. SONNEBORN†

DEPARTMENT OF ZOOLOGY, INDIANA UNIVERSITY

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Analysis of the determination and inheritance of antigenic traits in the unicellular animal, *Paramecium aurelia*, shows that cells which are identical in genic constitution may yield, under identical conditions of culture, progeny that are persistently diverse with respect to these traits, in spite of the fact that determination of the traits is ultimately traceable to the action of genes. As comparable persistent diversities among cells presumably identical in their genes arise normally in the development of higher organisms, the mechanisms discovered in Paramecium may be of general significance.

The present paper is the third of a series setting forth results obtained in a study of variety 4 of *P. aurelia*. The first two papers,[1, 2] in which the earlier literature is reviewed, may be briefly summarized as follows. In stock 51, four antigenically diverse hereditary types (A, B, C and D) have arisen; each of these is immobilized only by its corresponding dilute antiserum, not by dilute antisera against the other three types. Exposure of animals of any one of these types to its immobilizing antiserum for a short time is followed by transformation of antigenic type in high proportions of the exposed animals. These changes are from one to another of the four types, A, B, C and D. The transformed animals yield cultures that remain permanently true to their new type through both vegetative reproduction and fertilization. When interbred, the four antigenic types (both those that arise spontaneously and those that result from experimental transformations) manifest no segregation in the F_2 generations, indicating the absence of gene differences among them. Antigenic type strictly follows the cytoplasm, indicating its cytoplasmic control. Other points and the interpretations will be deferred until after the new data have been presented.

New Materials and Extension of Previous Results to Them.—Two new antigenic types, E and G, have arisen in the same stock 51 and immobilizing antisera have been prepared against them. A few cultures do not react to any available antisera, so one or more antigenic types remain to be identified. In another stock (No. 29) of variety 4, six diverse antigenic types have been isolated and identified and one or more remain to be investigated. Of the six known types, four are immobilized by the same antisera that immobilize types A, B, C and D, respectively, in stock 51. They may therefore be designated by the same symbols, though there is evidence that at

least some pairs of corresponding antigenic types in the two stocks are not absolutely identical. Thus far, no culture in stock 29 corresponds to type E or G of stock 51. On the other hand, two types, F and H, against which immobilizing antisera have been prepared, have arisen in stock 29 and thus far no culture in stock 51 corresponds to them.

All the new antigenic types (E, F, G and H), as well as the stock 29 homologs of types A, B, C and D, have been exposed to immobilizing antisera, in the way previously reported[1, 2] for types A, B, C and D of stock 51, in order to ascertain whether hereditary transformations of antigenic type could be induced. Using this method, large proportions of the animals (sometimes up to 100%) of every one of the types in both of the stocks can be transformed to other types. All transformations induced by exposure to antiserum fall within the spectrum of types characteristic of the stock employed (A, B, C, D, E and G in stock 51; A, B, C, D, F and H in stock 29). Even within these spectra, however, there are curious limitations. For example, in stock 29, Type D seldom transforms directly to Type F after exposure to anti-D serum; but it readily transforms to Type B which in turn can readily be transformed to Type F by exposure to anti-B serum. Any type in the stock 29 spectrum can be transformed to Type F by not more than two steps. Moreover, the transformations are reversible. Thus, if Type A of stock 51 has been transformed to Type B by exposure to anti-A serum, the resulting Type B cultures can be transformed back to type A by exposure to anti-B serum. As with transformations to Type F, reversals of type may sometimes require a two-step transformation. Transformation thus involves no complete loss of any antigenic possibility.

Interconvertibility of the types within a stock shows that each type possesses, in addition to the antigen that characterizes it, the capacity to produce the antigens characteristic of all the other antigenic types within the spectrum of the stock to which it belongs. Each hereditary antigenic type has therefore been defined[1, 2] as having a primary antigen, the kind involved in its immobilization by antiserum, and a number of secondary or potential antigens, the remaining kinds in the spectrum of the stock to which it belongs. One of the secondaries becomes the primary when transformation occurs spontaneously or is induced. In the earlier papers[1, 2] the various secondaries were held to be present in relatively small amount because relatively small amounts of specific antibodies against them were frequently detected in the antisera. In subsequently obtained antisera, however, antibodies against secondaries have been detected less frequently. While presence of antibody in a serum is evidence for presence of corresponding antigen in the paramecia used for injection, absence of antibody in the serum is not sufficient evidence for absence of the corresponding antigen. Secondary antigens appear therefore to be at least sometimes actually, and always potentially, present.

Control of the Direction of Transformation.—Our previous papers[1, 2] reported no control as to which new type or types would arise following exposure to antiserum. Theoretical considerations, set forth below, led to testing the possibility of *directing* transformation by combining exposure to immobilizing antiserum with exposure to certain other conditions, of which only diverse temperatures and amounts of food have thus far been explored. The results were dramatic. Decrease of temperature and decrease in amount of food have similar effects and together they have effects greater than either has alone. The full results, carried out on all 12 type-stock combinations with temperature and food varied separately, will be presented elsewhere. They are well illustrated by the following example. When Type D of stock 29 is exposed to anti-D serum, 94% of the animals transform to Type B if exposure and subsequent growth for a day or two are carried out at 32° in the presence of excess food; but 96% transform to Type H if the same experiment is carried out at 20° with enough food to permit only one fission per day. Similar control of the direction of transformation has been achieved with the other antigenic types.

In view of these results, further theoretical considerations led to the prediction that similar directed transformations should be possible, but more slowly, by employing diverse temperatures and food supplies, without subjection to immobilizing antiserum. Tests fulfilled the prediction. Extensive experiments, which will be presented in full elsewhere, are well illustrated by the following examples. In stock 51, without exposure to antiserum, at 32° Type A remains constant, but types B, C, D and E all transform to Type A; while at 12° Type B remains constant and other types transform to Type B. The transformations at 12° are slower and less regular than transformations at 32°. The effect of food supply is shown conspicuously by animals of Type C in stock 51, which, at the standard temperature of 27°, remain constantly Type C if provided daily with enough food for only one fission, but transform to Type A if provided with an excess of food. Our earlier difficulty[2] in maintaining stable cultures of this type resulted from providing them with too much food.

The facility with which antigenic types can be transformed by exposure to antiserum, to diverse temperatures and to diverse food supplies (perhaps also to other as yet untested conditions) is in marked contrast to the stability of all the types, regardless whether of spontaneous or induced origin, under standard conditions of culture at 27° with enough food added daily to permit one fission. Under these conditions, each type, regardless of origin, is hereditary, maintaining itself constantly through both vegetative and sexual reproduction.

Relation of Cytoplasmic to Genic Factors in Determination of Antigenic Type.—As set forth above, diverse antigenic types in the same stock are alike in genes and diverse in cytoplasm. The question arises as to whether

the antigenic differences between stocks are determined in the same way as antigenic differences within a stock. The one stock difference thus far analyzed is the capacity to manifest antigenic Type F, which exists in stock 29, not in stock 51.

The two stocks were crossed and the F_1 generation was allowed to produce an F_2 generation by autogamy (self-fertilization in unpaired animals). The antigenic types appearing in F_1 and F_2 are not in themselves of primary interest and those details will be presented elsewhere. Here we are concerned primarily with whether each F_2 clone could or could not be transformed to Type F (if it was not initially Type F), when subjected to the conditions known to favor transformation to that type. Half of the F_2 clones were Type F or were transformable to Type F and the remaining F_2 clones could not be induced to yield Type F. This 1:1 segregation in the F_2 occurred regardless of whether they were derived from F_1 clones with stock 29 cytoplasm or with stock 51 cytoplasm and regardles of whether there had or had not been exchange of cytoplasm in the initial cross between the two stocks. Apparently, therefore, the results are independent of F_1 cytoplasm. On the other hand, a 1:1 ratio among clones obtained by autogamy is the result required[3] when dealing with segregation of one pair of allelic genes.

The two classes of F_2 clones were then backcrossed to the parent stock of opposite class and autogamy was obtained in the backcross clones. Both sets of experiments yielded again the same 1:1 segregation. For the three experiments together, the totals were 86 clones capable of yielding Type F and 95 clones incapable of yielding Type F. The capacity to yield clones of Type F thus depends on a single chromosome, and presumably a single gene, present in stock 29 and lacking in stock 51. This gene determines not the realization of Type F, but only the capacity to realize it under definable conditions. If the other antigens show similar mechanisms, it may be concluded that the genes control the antigenic possibilities, while the cytoplasm controls which of the possibilities is realized and perpetuated.

Summary and Interpretations.—(1) The antigenic types are hereditary under standard conditions. (2) Diverse antigenic types within a stock are alike in genes, but differ in cytoplasm. (3) Capacity to develop antigen F depends upon a gene; hence, cytoplasmic control of antigenic diversity within a stock is ultimately gene-dependent. (4) Hereditary transformations within the spectrum of antigenic possibilities of a stock are readily brought about by environmental conditions (specific antiserum, temperature, food supply). (5) Approximately 100% hereditary transformation in either of two alternative directions may be produced by specifiable conditions; hence, the gene-dependent cytoplasmic control of antigenic diversity within a stock is also environment-dependent. Unlike the well-known examples in higher organisms of alternative phenotypes being de-

pendent upon the conditions during development, the effects of external conditions on the phenotype in Paramecium persist throughout all subsequent generations, asexual and sexual, under standard conditions.

The foregoing relations may be interpreted on the hypothesis that each antigen is, or is determined by, a plasmagene producible under the action of a gene or genes; and that the diverse plasmagenes compete with each other, the competition being influenced by environmental conditions and by the relative cellular concentrations of the various plasmagenes. On this hypothesis, the constancy of all antigenic types is due to the multiplication of all the plasmagenes at the same rate of one duplication per day under standard conditions of culture (i.e., the rate of cell multiplication under these conditions). Antibody merely facilitates transformation by suppressing or retarding multiplication of the antigen with which it combines (see Beale)[4] Then one of the other plasmagenes, whichever is best adapted to the prevailing conditions, replaces the suppressed one. Transformations following cytoplasmic exchange at conjugation are set in motion as a result of lowering the concentration of the original plasmagene and raising the concentration of the acquired plasmagene, the change in proportions providing a new competitive situation.

One of the main alternatives to the plasmagene hypothesis is an hypothesis of variable gene activity[5] in which the activity of each antigen gene varies directly, up to a certain limit, with the cellular concentration of the antigen it controls. To fit this hypothesis to the fact, however, a number of improbable assumptions are required. Moreover, when control of the direction of transformation was achieved on the basis of predictions suggested by the plasmagene hypothesis, a further improbable assumption had to be made to reconcile the new facts with the variable gene activity hypothesis. Although further critical tests distinguishing between these hypotheses remain to be made, the relative simplicity and reasonableness of the plasmagene hypothesis, together with its proved predictive value, justify its tentative adoption.

Relations to Other Work.—Evidence for gene-initiated plasmagenes in variety 4 removes the main difference previously held[6] to distinguish two groups of varieties of *P. aurelia*. Of the three kinds of traits—killer, antigens and mating types—that show cytoplasmic control in variety 4, two have now been analyzed: in one (killer trait) the cytoplasmic factor is *not* gene-initiated; in the other (antigens) the plasmagenes *are* gene-initiated. Counterparts of both mechanisms are in the literature: no evidence of gene-initiation has been reported for the CO_2 "genoid"[7] in Drosophila or for the plasmon[8] of plants; but gene-initiation of plasmagenes is indicated[9] for adaptive enzymes of yeast (see also reference 10). The large size and staining properties of the killer cytoplasmic factor kappa are unique[11] among the cytoplasmic factors in Paramecium; the failure of genes to

initiate it may likewise be unique. At least, gene-initiated plasmagenes can be more readily assimilated into current genetic knowledge and theory which demand ultimate gene control of hereditary capacities.

The cells of a multicellular organism are like the cells of a clone of unicellular organism in their presumed identity of genes. Nevertheless, in both the Metazoan and the Protozoan persistently diverse types of cells arise. In Paramecium, our analysis indicates that persistent diversity of traits may arise as a result of environmentally produced quantitative differences among a series of alternative plasmagenes. The same mechanism may therefore be suggested as the basis of cellular differentiation in higher organisms. If this turns out to be correct, control of cellular transformation in tissue cultures and in normal and abnormal development is indicated, and genetics has moved a step closer to its necessary ultimate fusion with experimental embryology.

* Contribution No. 388 from the Department of Zoology, Indiana University.

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‡ Under the title: "Genes, Cytoplasm, and Environment in the Control of Cellular Heredity."

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*THE PROCESS OF TRANSFORMATION OF ANTIGENIC TYPE IN PARAMECIUM AURELIA, VARIETY 4**

BY G. H. BEALE†

DEPARTMENT OF ZOOLOGY, INDIANA UNIVERSITY

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Introduction.—The diversities in antigenic type in *Paramecium aurelia*, variety 4, have been shown to be purely cytoplasmic in heredity (Sonneborn[1,2]), provided that attention is confined to animals of a single stock,

derived from a single wild ancestor. Since antigenic types breed true under certain conditions, continuing unchanged through both asexual and sexual reproduction, there must be some mechanism in the cytoplasm for the reproduction of the antigens or of their precursors. In this investigation an attempt is made to gather some data relevant to the problem by making a quantitative study of the antigens in paramecia under varying conditions, particularly during transformation of one antigenic type into another.

Measures of Antigens and Antibodies.—It has been shown by Masugi[3] that paramecia, when in sufficient quantity, are able to remove the paralyzing antibodies from an antiserum, rendering it ineffective against any fresh animals. The ability to absorb antibody from a standard solution may therefore be used as a measure of the antigen in an animal.

As a measure of antibody, the time taken for a paramecium to be immobilized when placed in a large excess of solution containing the antibody, can be used. Data in table 1 show that the reciprocal of the immobilization time is roughly proportional to concentration of antiserum for solutions immobilizing in 20 minutes or less, but that with weaker solutions, the immobilization time increases more steeply. Within a stock, the immobilization time, under standard conditions, is uniform; but animals from different stocks, though paralyzed by the same antiserum, may have significantly different immobilization times with the same concentration of antibody.

TABLE 1

RELATION BETWEEN IMMOBILIZATION TIME AND CONCENTRATION OF ANTISERUM D-47 AT 25°C.

DILUTION	IMMOBILIZATION TIME (MINUTES)		
1/200	4	5	4
1/400	10	10	10
1/800	21	22	22
1/1600	53	70	50
1/3200	>120	>120	>120

Each column represents a different sample; and each figure represents mean of 10 observations.

Some preliminary experiments were made on the rate of absorption of antibodies by paramecia. A known number of animals was placed in a given volume of suitably diluted antiserum, samples were removed from time to time, and centrifuged. The strength of antibody in the centrifuged sample (i.e., in the supernatant) was determined by putting a few fresh paramecia into it, and comparing their immobilization times with those of animals put into unabsorbed antiserum. The amount of antibody absorbed by the animals, expressed as a fraction of the amount originally present in the solution, could be then estimated. Table 2 shows some typical results.

It is seen that the rate of absorption is rapid at first, but soon slows down; and that the amount finally absorbed is roughly proportional to the number of animals.

Measure of Antigens during Transformation.—The antigenic type has been found to remain constant if the paramecia are provided with sufficient food for only one fission per day at 27°C. (Sonneborn[4]). Under these conditions the rate of production of antigens parallels the growth rate of the animals. During transformation of one type into another, however, there is a loss of one antigen and a simultaneous gain of a new one, and therefore the transformation period is a profitable time for the study of antigen production. As described by Sonneborn[1,2] transformation of Type D (stock 51) to Type B, is readily accomplished by treating the D animals with their paralyzing antiserum, subsequently placing the animals in culture fluid, allowing them to recover and pass through a few fissions.

TABLE 2

ABSORPTION OF ANTIBODIES FROM SERUM D-47 (DILUTION 1/500) BY TYPE D PARAMECIA

ABSORPTION PERIOD (MINUTES)	1350 ANIMALS PER CC. (1)	(2)	4050 ANIMALS PER CC. (1)	(2)	12,150 ANIMALS PER CC. (1)	(2)
0	11	0	11	0	11	0
15	..	..	..	..	19	0.42
30	..	..	16	0.31	26	0.58
60	12.5	0.12	16.5	0.34	30	0.63*
120	17	0.35	21	0.48	50	0.78*
180	16.5	0.34	20	0.45	..	...
300	17	0.35	..	..	..	...

Explanation of columns: (1) Immobilization time (minutes) of samples of solution after absorption. (2) Calculated proportion of antibody removed from solution during absorption assuming inverse linear relation between concentration of antibody and immobilization time.

* Owing to long immobilization time, these figures are approximate only.

It was first of interest to determine whether the serum treatment directly produced the transformation—i.e., whether paramecia on being removed from the serum (after treatment for two hours or less) had lost their D-type antigens and replaced them with B-type ones. This was found not to be so. Animals which had been immobilized by anti-D serum (though not with such a severe dose as to prevent subsequent recovery), could still absorb D-type antibodies from a solution, though to a lesser extent than normal D animals, as shown by the following experiment. The immobilization time of antiserum D-47 at dilution 1/800 was found to be 13 minutes. After absorption by 40,000 D animals for 20 minutes in 2 cc. of this solution (immobilizing all the animals), all detectable antibodies were found to be removed from the solution. The 40,000 immobilized animals, after being washed, were then placed in a fresh 2 cc. serum and after 30 minutes

had increased the immobilization time of the solution from 13 to 16 minutes, thus demonstrating that immobilized animals were still capable of absorbing antibody. After further washing and placing in fresh 2 cc. serum, the 40,000 animals were finally incapable of absorbing any more antibody in two hours. Thus the immediate effect of the serum treatment was to reduce the capacity of the animals to absorb anti-D antibodies.

It is not however necessary to immobilize the paramecia in order to bring about the transformation D→B, for even a mild serum treatment, sufficient only to effect a slight retardation of the animals, yields a high degree of transformation. With animals thus treated, it is easy to test their reaction to both anti-D and anti-B sera immediately after treatment, before recovery has taken place; such animals have always been found to be immobilized by anti-D but completely unaffected by anti-B serum. Thus immediately after treatment there are no signs of the impending transformation.

Transformation of antigenic type takes place only when a period of growth follows the serum treatment. A number of stages may be recognized in the transformation process, as follows:

1. Paramecia immobilized by anti-D; unaffected by anti-B (normal D animals).
2. Paramecia immobilized by anti-D; retarded by anti-B.
3. Paramecia retarded by anti-D; retarded by anti-B.
4 Paramecia retarded by anti-D; immobilized by anti-B.
5. Paramecia unaffected by anti-D; immobilized by anti-B (normal B animals).

The length of the various stages depends upon the strength of the initial serum treatment, i.e., the stronger the treatment, the sooner transformation begins and ends. Thus in one experiment paramecia of Type D were subjected for half an hour to anti-D serum diluted 1/800, which thoroughly immobilized the animals. After treatment the animals were washed and immediately put into culture fluid. With this treatment, stage 1 lasted until about five hours after the end of the treatment, and the whole process of transformation was substantially complete in 15 hours. By this time all the animals had passed through one fission, and about one half had passed two fissions. The number of clones transformed after 15 hours was 59 out of a total of 94; further growth for 12 hours (i.e., 27 hours in all) resulted in changes in only two more cultures, which produced mixtures of transformed and untransformed animals.

With a much weaker treatment however (half an hour of anti-D serum diluted 1/6400), stage 1 lasted a minimum of eight hours, and by 15 hours only 6 out of 76 cultures had progressed as far as stage 4. But after 27 hours, a further 29 cultures had produced transformed animals. Thus, with

the weak treatment, the time at which transformation of individual animals began and ended was much more variable than with the strong treatment, though almost always later.

The necessity for active metabolism during transformation was demonstrated by placing serum-treated animals in boiled tap water for five days. Animals which had been given a weak serum treatment (though sufficient to induce transformation in animals subsequently fed and grown), did not transform at all when kept in boiled water. A stronger serum treatment, followed by starvation in water, resulted, after 20 hours, in the development of animals which, while still liable to immobilization by anti-D, were at the same time more or less retarded by anti-B serum. Starved animals, however, even after five days, never lost their reactivity to anti-D. Presumably the starved animals contained enough reserve materials to allow a limited metabolism, sufficient for partial transformation.

TABLE 3

EFFECT OF STARVATION ON TRANSFORMATION

	TRANSFORMATIONS AFTER 6 TO 8 FISSIONS					
	ANIMALS GROWN IMMEDIATELY AFTER TREATMENT		ANIMALS STARVED 24 HOURS, THEN GROWN		ANIMALS STARVED 5 DAYS, THEN GROWN	
TREATMENT	TRANSFORMED CLONES	UNTRANSFORMED CLONES	TRANSFORMED CLONES	UNTRANSFORMED CLONES	TRANSFORMED CLONES	UNTRANSFORMED CLONES
1	29	0*	19	10	9	21
2	30	0	14	16	8	22
3	28	2	10	18	2	28
4	14	13	2	28	0	30

Treatments: 15,000—51 D animals in 1 cc. culture solution plus the following amount of D-47 serum diluted 1/400 for two hours: 1—1 cc.; 2—0.5 cc.; 3—0.25 cc.; 4—0.125 cc.

If serum-treated animals were starved, and subsequently fed, transformation was completed, though the proportion of transformed animals finally obtained became progressively less as the period of starvation became longer. Data in table 3 illustrate this. The most probable explanation of this gradual loss in ability to transform following starvation is a slow dissociation of the antigen-antibody complex while the animals are in water, though no direct evidence of this has been obtained.

Conclusions and discussion.—As a result of these experiments, the following conclusions can be made:

1. Treatment of D-type paramecia from stock 51 with dilute anti-D serum for two hours or less, produces animals with a reduced capacity to absorb D-type antibodies. Immediately after treatment, however, the animals can still be paralyzed by anti-D serum only, and are unaffected by anti-B serum.

2. When a period of active growth at 26° follows serum treatment, some

of the animals gradually gain an increasing amount of B-type antigen, and at the same time their reaction to anti-D serum becomes progressively weaker and finally disappears.

3. The times at which transformations from Type D to Type B begin and end are related to the strength of the original serum treatment, i.e., to the amount of antibody absorbed by the treated animals. With a strong, immobilizing treatment, transformation is complete by 15 hours after treatment (or slightly less than two fissions); but with a very weak treatment, completion of the process may vary from 15 to 24 hours or more.

4. Transformation is completed only in the presence of food.

Sonneborn[2,4] has put forward a theory according to which antigen type in *P. aurelia*, variety 4, depends upon competition between plasmagenes. A given stock, or more exactly a given genotype, is capable of producing, under diverse conditions, any one of a number of antigen types (at least six in stock 51). The type actually selected, however, depends upon the results of the plasmagene competition.

The present results strongly support this theory. Further, the indications are that treatment by anti-D serum partially or completely suppresses the further production of D antigens, from which it would appear that the plasmagenes are the antigens themselves.

Finally, attention should be drawn to the striking similarity between the mechanism of antigen transformation here described, and the enzymatic adaptations in yeasts,[5,6] and bacteria.[7]

* Contribution No. 389 from the Department of Zoology, Indiana University.

† Present address: Institute of Animal Genetics, Edinburgh. Investigation carried out during tenure of a Rockefeller Fellowship, and aided by grant from Rockefeller Foundation for work in genetics at Indiana University.

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